Dangerous Liaisons between Beta-Amyloid and Cholinergic Neurotransmission

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Abstract: The review examines the multifaceted interactions between cholinergic transmission and beta-amyloid suggesting a continuum in the action of the peptide that at low concentrations (picomolar-low nanomolar) may directly stimulate nicotinic cholinergic receptor while desensitizing them at increasing concentrations (high nanomolar-low micromolar). In addition high beta amyloid concentrations may reduce the synaptic release of several neurotransmitters, including glutamate, aspartate, GABA, glycine and dopamine, when the release is elicited through cholinergic stimulation but not following depolarization. The effect of beta-amyloid has been observed both *in vitro* and *in vivo* in at least three different brain areas (nucleus accumbens, striatum, hippocampus) suggesting that the peptide may exert some general effects even if not all the brain areas have been evaluated. In turn the activation of cholinergic receptors may affect the amyloid precursor protein processing diverting the metabolism toward non-amyloidogenic products. These actions, dissociated from those described in the case of high beta-amyloid concentrations leading to neurotoxic oligomers, may participate to cause dysfunctions in the neurotransmitter activity, in turn leading, at least from a theoretical point of view, to early neuropsychiatric disturbances in the disease. Complexively these observations underscore novel relationships between two main players in Alzheimer's disease pathogenesis that are beta-amyloid and cholinergic transmission. Also emerges the inherent difficulty of targeting beta-amyloid in a context in which the peptide exerts several actions beyond neurotoxicity.

Keywords: Alzheimer's disease, beta-amyloid, cholinergic neurotransmission, nicotinic receptors, neurotransmitters.

INTRODUCTORY REMARKS

Alzheimer's disease is characterized by several neurochemical defects, among which two have been almost universally described as occurring during the mild to moderate phase, that are beta-amyloid deposition and cholinergic neuronal loss. The former is thought to precede the loss of cholinergic terminal markers. With disease progression also a massive tau protein deposition takes place according to an ordered pattern starting from the entorhinal cortex and then involving an increasing number of brain regions [1]. These events have been substantially studied and discovered as independent phenomena; however several mutual interactions are present. Within this context the present review examines the interplay between two of the three main players: amyloid and cholinergic transmission. The reasons for this choice are that both in the older and in the more recent theories on AD pathogenesis (as reviewed in [2-4]) beta-amyloid is considered an upstream event and, in addition, literature data suggest functional interactions between these two elements besides a putative amyloid-induced degeneration of cholinergic terminals.

The August 2012 announcement that the clinical testing of the Alzheimer's disease (AD) drug Bapineuzumab (a monoclonal antibody directed against beta-amyloid) has been halted after two failed clinical trials has underscored once more the fact that we do not know for sure what causes the disease and what are the check points to target in order to reverse, halt or at least slow down the progression of the disease (for a review on new interventions see [5]). Within this context, beta-amyloid cannot be, however, yet disregarded as an invalid theory, but needs to be settled in a more complex context, considering not only its accumulation but also its biological effects within the time frame of the disease course. While little or no correlation has been found between high levels of beta-amyloid in the brain and cognitive imparment in AD [6] cholinergic

impairment do correlate with the characteristic symptoms of the disease [7, 8]. These symptoms are not alleviated by amyloid-based strategies (as for example immunization, [9]), but they may be ameliorated by cholinergic-based interventions [10]. Presently three of the four approved drugs for AD treatment are indirect central cholinergic agonists based on the central inhibition of the acetylcholine degrading enzymes (acetylcholinesterase, AChE) and on the observation of a prominent degeneration of cholinergic neurons. These drugs, acetylcholinesterase inhibitors (AChE-I), are effective in reducing the symptoms and, according to some authors, also in slowing down the disease progression in a significant proportion of patients with mild to moderate disease, but not in the preclinical stages [11-13] pointing to the importance of targeting the cholinergic system in at least a proportion of patients. Accordingly, it seems important to study the amyloid-cholinergic relationship, in order to understand whether the mutual interactions between these two main actors in AD pathogenesis may open new perspectives in drug treatment or at least explain the limitations of the current interventions and the failure so far of the amyloid targeted therapies.

Four are the main domains explored by the present review: a) bimodal relationships between cholinergic transmission and Amyloid Precursor Protein (APP) processing; b) direct interactions between beta-amyloid and nicotinic receptors; c) direct interactions between amyloid and AChE enzyme; d) mutual amyloid-nicotinic interactions in neurodegeneration, mostly, but not exclusively, elicited by excess amyloid; e) mutual interaction of nicotinic stimulation and beta-amyloid in the modulation of neurotransmitter release from synaptic terminals, an event which may occur before neurodegeneration.

CHOLINERGIC TRANSMISSION IN AD BRAIN, A BRIEF SUMMARY AND SOME CONSIDERATIONS

The most consistently reproduced finding of the 1976 paper by Davies *et al*. is a profound reduction in the activity of the acetylcholine (ACh)-synthesizing enzyme, choline acetyltransferase (ChAT), in the neocortex of AD patients, which correlates positively with the severity of dementia [7]. Reduced choline uptake, ACh release

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and loss of cholinergic neurons from the basal forebrain region further indicate a selective presynaptic cholinergic deficit taking place in the hippocampus and in the neocortex of brains of individuals with AD [14]. Cholinergic neurons in other brain regions such as in brain stem and in striatum are either spared or affected only in late stages of the disease. The loss of basal forebrain cholinergic neurons has stimulated extensive studies of ACh receptors in the brains of individuals with AD. Among the five distinct muscarinic receptor subtypes, M2 receptors, most of which are located on presynaptic cholinergic terminals, are reduced in the brains of individuals with AD. For the nicotinic receptor family, high-affinity nicotinic binding sites are markedly reduced in the hippocampus and cortex of postmortem brains of individuals with AD, and these observations have been confirmed by *in-vivo* positron emission tomography by the Karolinska group since the mid nineties [15].

The neat 2005 paper by Perry *et al*. confirmed in a small but very well controlled set of cases with anatomopathological confirmation of the diagnosis the robust loss of cholinergic terminals in the brain of AD and mixed AD-vascular dementia (VD) patients but not in pure VD [16]. Cholinergic deficits are shared by the Parkinsonism associated dementia and in Lewy body Dementia (LBD) [17], which may also benefit of acetylcholinesterase inhibition, even if more data are needed to make this point consistent [18]. Notably, there is no amyloid accumulation in LBD. On the other hand, another dementia, associated with a prominent derangement in tau but not amyloid protein aggregation, the frontotemporal dementia (FTD), is associated with deficiencies in the serotonin and dopamine neurotransmitter systems, whereas the cholinergic transmission appears relatively intact [19]. It is tempting to speculate that the changes in amyloid and tau deposition in AD may converge at a certain time point in AD brain determining it peculiar neurochemical signature or that these independent events may contribute but are not direct causes for the cholinergic terminal degeneration. An hypothesis explaining the degeneration of cholinergic neurons in AD has been proposed: a dysregulation of the nerve growth factor (NGF), i.e. an impairment of the conversion of the precursor form of NGF (pro-NGF) to the mature form of NGF (m-NGF) in addition to an increased degradation of m-NGF. The subsequent decrease in the level of m-NGF would lead to cholinergic atrophy since basal forebrain cholinergic neurons are highly dependent to this neurotrophin for the maintenance of their phenotype and synaptic integrity [20, 21]. Interestingly, the administration of beta-amyloid oligomers to naïve animals has been shown to cause similar alterations in the NGF metabolic pathway but these effects are mediated by amyloid-induced inflammatory processes [22].

Altogether, these observations further stresses the importance of the studies examining amyloid-cholinergic transmission interactions and their development during the disease progression, to appreciate their functional consequences, besides neurodegeneration.

APP PROCESSING AND CHOLINERGIC SYSTEM: BIMODAL RELATIONSHIPS

The processing of APP has been reviewed elsewhere [23-25]. In brief: APP is a type I transmembrane protein with a large extracellular domain, a membrane anchoring domain and a short intracellular C-terminal tail. The mature form of APP is processed by at least two distinct proteolytic pathways.

One pathway involves cleavage by the enzyme alpha-secretase, which cuts APP within the beta-amyloid sequence, thereby preventing the formation of beta-amyloid. This step produces a secreted form of APP (sAPPalpha) and a C-terminal fragment, which remains associated to the membrane.

A second pathway involves cleavage of APP by an enzyme referred to as beta-secretase. Beta-Secretase cleaves APP at the N-terminal side. Cleavage results in the production of a N-terminally truncated form of APP (sAPPbeta) that is released from the membrane and a C-terminal membrane-associated fragment (C99 or

beta-CTF). Following cleavage by alpha or beta secretase, the gamma-secretase complex cleaves, inside the membrane, the remaining C-terminal fragments of APP, C83 and C99, via a mechanism referred to as regulated intramembrane proteolysis. The combined action of beta and gamma-secretase leads to the production of beta-amyloid. Notably the processing of APP can be modulated by various receptors for neurotransmitters and by drugs, including those acting upon cholinergic receptors, both muscarinic and nicotinic and also by cholinesterase inhibitors [26-31]. In particular the latter have been shown to promote the non-amyloidogenic metabolism of the precursor protein. Even if this phenomenon has been shown to occur also at clinical level [32] following cholinesterase inhibition it is not clear whether or not it contributes to the clinical response, although the recent failure of the direct anti-amyloid interventions argue against this possibility. On the other hand, the possibility that the cholinergic system may regulate APP processing deserves attention, since amyloid itself can act as a regulator at cholinergic synapses (see below) and the various products of APP cleavage may regulate various cellular activities [23].

Activation of muscarinic acetylcholine receptors (mAChRs) promotes the non-amyloidogenic pathway, and in the majority of the cases, concomitantly reduces beta-amyloid production. Particularly, these effects are selectively exerted by the activation of mAChRs subtypes that are coupled to a downstream signalling pathway involving protein kinase C (PKC), such as M1 and M3, but not M2 and M4 receptor subtypes [33-36]. On the other hand, stimulation of other phospholipase C-coupled receptors, i.e. bradykinin [37], thrombin [38], metabotropic glutamate [39], and serotonin $5-HT_{2a}$ and $5-HT_{2c}$ receptors [40], also increases the soluble alphaAPP secretion. Moreover, different studies (reviewed in [29, 41] showed that the direct activation of PKC by means of phorbol esters also promotes the non-amyloidogenic pathway and decreases beta-amyloid release.

The muscarinic control of APP processing has also been evaluated *in vivo*. In a transgenic mouse model of AD (APPSwedish/ Indiana), genetic deletion of M1 mAChRs (APPSwedish/Indiana x M1KO mice) results in increased levels of pathogenic beta-amyloid peptides in brain, as well as increased accumulation of amyloid plaque pathology. Transgenic expression of the M1 mAChR on the M1 mAChR knock-out background rescued the observed phenotype, indicating that endogenous activation of the M1 mAChR is sufficient to shift APP processing towards the nonamyloidogenic route [42]. In light of the cholinergic impairment in AD brains and in light of the role of mAChRs on APP processing toward the non-amyloidogenic route, different muscarinic agonists have been evaluated as possible treatment for AD patients. However, clinical studies with unselective muscarinic agonists but also with selective M1 agonists were discontinued due to a variety of intolerable side-effects (e.g. hypotension, sweating, bronchoconstriction) [43].

Some studies have investigated the role of nicotinic acetylcholine receptors (nAChRs) on APP processing. In PC12 cells (a rat pheochromocytoma cell line), Kim and colleagues first showed that nicotine treatment increases the release of sAPPalpha (considered trofic and neuroprotective), without affecting the expression of APP mRNA, through a mechanism involving a Ca^{2+}-dependent signalling. This effect was attenuated by co-administration of mecamylamine, a non-selective antagonist of nAChRs, indicating that this was a receptor-mediated effect [44]. Notably, galantamine, an AChE inhibitor but also an allosteric potentiator of nAChRs also increases the secretion of sAPP from human SH-SY5Y neuroblastoma cells through the activation of nAChRs [31]. In adition, in brain of rodent AD models, galantamine facilitated beta-amyloid clearance [45].

Both alpha4beta2 and alpha7 nAChRs subtypes seem to be involved in the nicotinic regulation of APP metabolism. In human SH-EP1 cells (derived from SK-N-SH human neuroblastoma), stably transfected with both human alpha4beta2 nAChRs and human

APP, application of either nicotine or epibatidine (a nicotinic agonist) decreased the secretion and intracellular accumulation of beta-amyloid without affecting the expression of APP [46]. Mousavi and Hellström-Lindahl [47] observed that nicotine increased the release of sAPPalpha whilst decreasing beta-amyloid levels in SH-SY5Y cells expressing the alpha7 nAChRs subtypes and this effect was blocked by mecamylamine.

Altogether, cholinergic stimulation of both muscarinic (M1, M3) and nicotinic (alpha4beta2 and alpha7) receptors contribute to regulate APP processing promoting the production of sAPPalpha and decreasing the secretion of beta-amyloid.

However the significance of this regulation within the physiology of the control of peripheral cholinergic signalling or in relation to the control of AD pathology is largely unknown.

DIRECT EFFECTS OF BETA-AMYLOID ON NICOTINIC RECEPTORS

Beta-amyloid may directly activate cholinergic nicotinic receptors, at very low concentrations interacting with a specific ligand binding domain in the alpha7 nicotinic receptors as also demonstrated by site directed mutagenesis [48]. These data add to the concept of a direct neuromodulatory role of beta-amyloid played at nicotinic receptors and lead to speculate that the increasing amyloid concentrations during the disease would perturb such function. The relationship between nicotinic cholinergic receptors and beta-amyloid has been recently carefully reviewed by Parri *et al* [49] (see also [50]). Of particular interest appears to be the already mentioned interaction between the alpha7 nicotinic receptor and beta-amyloid. The interactions appear to be complex and depending upon the cellular milieu, the choice between beta-amyloid 1-40 and 1-42, the concentration and the aggregation status of beta-amyloid and the time of exposure. Indeed beta-amyloid isoforms and oligomers of increasing molecular size may have different biological actions in a continuum from physiology to pathology, determining loss and gain of function along the course of the disease [51].

The number of conditions and experimental models evaluated in the literature, the uncertainty in several of the published papers about the exact molecular species of amyloid adopted prevent excessive generalization of the results, however definite trends emerge. In particular it appears that: a) both beta-amyloid 1-40 and 1-42 bind to the alpha7 nicotinic receptor, although beta-amyloid 1-42 is more effective in competition binding studies compared to beta-amyloid 1-40; b) the affinity for amyloid of the alpha7 receptor is 100-5000 times higher than that of the alpha4beta2 receptor which, however, cannot be disregarded as a target for the higher concentrations; 3) there are both competitive and non competitive interactions [49].

The interaction between beta-amyloid and the nicotinic alpha7 receptor can lead both to activation, and inactivation, mostly depending upon the concentration of the peptide and the time of exposure. Notably, *in vivo* experiments by Puzzo *et al.* [52, 53] have shown that picomolar beta-amyloid concentrations activate signal transduction cascades associated with neuroprotection, synaptic plasticity and learning and memory in an alpha7 dependent manner. On the other hand beta-amyloid may activate (short term action)/deactivate (long term action) astrocyte alpha7 receptors in turn regulating neurotransmitters and inflammation mediators. Higher concentrations (nanomolar and above) or prolonged exposure lead to receptor inactivation. Activation may lead to the promotion of intracellular signals, including various kinases, mediating, among the others, neuroprotective effects, whereas inactivation may lead to inappropriate synaptic signalling, and neuronal degeneration in response to aversive stimuli. It has been speculated that with AD progression the accumulation of beta-amyloid and of its oligomers may lead to progressive inactivation of the receptors (due to the prolonged exposure and/or to the increasing peptide concentrations)

with consequent impairment of nicotinic cholinergic transmission through alpha7 receptors and neurodegeneration [49]. Indeed beta-amyloid content in the brain of non-AD subjects is in the picomolar range, whereas nanomolar quantities are found in AD brain [54-55]. The situation is even more complex since different beta-amyloid oligomer assemblies are observed in AD brains depending upon age of disease onset. In particular, the predominant oligomer assemblies detected are dodecamers, decamers and pentamers with different patterns of expression between early and late onset AD. Levels of pentamers significantly correlated with reduction in acetyltransferase activity in AD brain, whereas total amounts of beta-amyloid oligomers and decamers correlated negatively with nicotinic receptors, suggesting that distinct beta-amyloid oligomers may induce impairment of cholinergic transmission [56]. Moreover, a negative correlation between ^{11}C-Pittsburgh Compound B (PIB) positron emission tomography (PET) retention and levels of ^{3}H-nicotine binding at autopsy and interactions between fibrillar beta-amyloid and alpha7 nAChRs have also been observed [57, 58], further underlying the complexity of the scenario and supporting a role for nAChRs in the amyloid pathology. In particular, it has been suggested that agonists of the alpha7 nAChR can modulate the binding of beta-amyloid to the receptor. Interestingly, chronic nicotine exposure has been shown to upregulate nAChRs, thus reverting their loss in the disease, and to decrease the levels of soluble and insoluble beta-amyloid 1-40 and 1-42 in the human brain and in both the brain parenchyma and vessels of a mouse model of AD (the APP-Swedish mice) [59, 60]. All these observations, indicates that selective nAChRs agonist may represent disease-modifying treatments for AD. However,the development of nicotinic agonists was hampered by various problems such as poor selectivity for nAChR subtypes, quick adaptive responses of nAChRs, poor pharmacokinetics or excessive toxicity [61-63].

On the other hand, the hypothesis on the amyloid-alpha7 nAChRs interaction leading to peculiar aspects of AD disease, neurodegeneration and loss of cholinergic synapses, while interesting, does not fit all the observations. Indeed the time scale of the activation/inactivation switch seems to be much shorter than the one underlying disease development and progression. In addition Li *et al.* [64] have shown that the smallest beta-amyloid sequence inducing suppression of hipocampal long term potentiation (LTP) is the 31-35 sequence at nanomolar concentrations, apparently acting through alpha7 nAChRs activation. Moreover, beta-amyloid cannot be the sole player since degeneration of the basal forebrain cholinergic neurons takes place also in Lewy body dementia in which the accumulation of beta-amyloid has a pattern different from the one observed in AD [65].

While the literature on the direct interactions between beta-amyloid and the nicotinic receptors is relatively abundant, to our knowledge there are no published reports on direct effects of the peptide on muscarinic recognition sites. Rather, the activation of the latter may affect the processing of the amyloid precursor protein (see [33] for a recent review and the preceding paragraphs) decreasing beta-amyloid production. On the other hand beta-amyloid may act upon the intracellular molecular cascade activated by muscarinic receptors linked to PKC activation as shown in various experimental conditions [66-68].

Besides the effects of the interaction of beta-amyloid with nicotinic receptors on neuronal survival, it is of interest to investigate whether there are effects also on the direct neuron to neuron signalling at synaptic level. Such an action would be compatible with the localization of the nAChRs on presynaptic terminals as well as on postsynaptic elements and would argue in favour of short term functional effects of the peptide. The following paragraphs examine the consequence of beta-amyloid interaction with nAChRs both on the neuroprotective and on the neuromodulatory action, with a greater emphasis on the latter.

STIMULATION OF NACHRS AND ATTENUATION OF BETA-AMYLOID NEUROTOXICITY

There is a very rich literature showing that the stimulation of nAChRs attenuates cytotoxicity by activation of survival pathways involving signalling, such as the PI3K-Akt, JAK2/STAT3, MEK/ERK related to each other through intracellular pathways. These responses are neuroprotective versus various neurodegenerative stimuli and are mediated by alpha7 nAChRs. Recent data also show an alpha7 nAChRs mediated stimulation of beta-amyloid phagocytosis operating in microglia and promoting effective amyloid removal. All these phenomena may take place and accompany the progression of the illness as an originally defensive response until a full-blown neurodegenerative picture emerges. Interestingly, over expression of APP may reduce the expression of nAChRs. It is tempting to speculate that in such a case cholinergic terminals activity will be disrupted by a dual mechanism: a reduced expression of the nicotinic receptors and their desensitization due to an excessive production of beta-amyloid among the other products of APP metabolism. The specific degeneration of cholinergic neurons remains, however, unexplained. The progressive loss of cholinergic innervation is predicted to produce an accelerated neuronal degeneration and in turn treatment with indirect cholinomimetics such as cholinesterase inhibitors would sort neuroprotective effects, which are not evident in the human clinical setting even if demonstrated in animal models [69].

BINDING AND DIRECT EFFECTS OF BETA-AMYLOID ON ACHE

As described above, AD brain is characterized by the impairment in the cholinergic system. AChE-rich neurons and fibers are decreased in AD compared to normal brain [70]. The activity of both ChAT and AChE decreases with increasing severity of AD [71], to the point where in severe AD there is little synthesis and hydrolysis of ACh.

Despite the overall loss of AChE, *post-mortem* analysis of AD brain showed that AChE activity is increased around amyloid plaques very early in the process of amyloid deposition [72, 73], suggesting a possible interaction between this enzyme and beta-amyloid. AChE may directly interact with beta-amyloid promoting its assembly into amyloid fibrils with the formation of highly toxic beta-amyloid-AChE complexes (as reviewed by [74]). The neurotoxic effect induced by beta-amyloid-AChE complexes was higher than that induced by the peptide alone in both *in vitro* (hippocampal neurons) and *in vivo* (rats injected with the peptide in the dorsal hippocampus) experimental settings [75]. Also *in vivo* studies using double transgenic mice overexpressing both AChE and beta-amyloid support the hypothesis that AChE may play a role in AD pathogenesis [76].

On the other hand, Sberna *et al*. [77] found that chronic exposure (over 7 days) of neuronally differentiated P19 cells to soluble beta-amyloid 1-40 and 25-35 causes an increase in AChE activity and this effect was mediated by L-type voltage-dependent calcium channels. More recently, Chauhan and Siegel [78] have shown five-fold higher levels of AChE in the cortex of a transgenic mouse model for AD (the Tg2576 mouse) whose phenotype is characterized by the expression of high levels of beta-amyloid and, with increasing age, amyloid plaque deposition. Notably, it has been suggested a potential locus of interaction for beta-amyloid near the peripheral site of AChE, on the external surface of the enzyme near to its catalytic site [79]. In primary cortical neurons Fodero and colleagues [80] examined the mechanism underlying beta-amyloid enhancement of AChE activity and showed that beta-amyloid 1-42 is more potent than beta-amyloid 1-40 in its ability to increase AChE and that the induced increase in AChE by high molecular weight aggregates of beta-amyloid 1-42 (10μM) is mediated by a direct agonist effect on alpha7 nAChRs. In fact, alpha7 selective antagonists (i.e. methyllycaconitine) inhibited the beta-amyloid 1-42-mediated increase in AChE and, conversely, prolonged treatment (over 2.5 days) with alpha7 agonists (i.e. choline), but not with agonists selective for other nAChRs (i.e. epibatidine), mimicked the effect of the peptide on AChE.

Recent observations show that AChE can regulate APP metabolism and beta-amyloid production. In fact, Silveyra and collaborators [81] showed an interaction between AChE and presenilin-1 (PS-1, the active component of gamma-secretase complex) by reciprocal co-immunoprecipitation. In SH-SY5Y cells the same authors [82] also showed that tacrine, an AChE-inhibitor, decreased PS-1 levels and in parallel increased the secretion of sAPPalpha. In the same cellular model, AChE over-expression increases PS1 levels, whereas AChE knock-down with RNA silencing decreased PS1. Treatment of SH-SY5Y cells with beta-amyloid 1-42 triggered elevation of both AChE and PS1 levels and interestingly, the peptide-induced PS1 increase was abolished by AChE silencing [82]. Hence, the authors hypothesized that AChE may participate in a vicious cycle that enhances amyloidogenic APP processing. On the other hand, some of the observed results may also be due to indirect events such as an altered cholinergic signalling due to perturbation of the degrading enzyme activity/expression.

PRESYNAPTIC NACHRS REGULATING NEUROTRANSMITTER RELEASE AS TARGET FOR BETA-AMYLOID

nAChRs are widely expressed throughout the central nervous system and participate in a variety of physiological functions among which synaptic transmission and synaptic plasticity, particularly in the hippocampus and midbrain dopamine areas (for a review see [83, 84]. When nAChRs are expressed on presynaptic membranes their activation generally increases the neurotransmitter release. At postsynaptic level, nAChRs initiated calcium signals and depolarization activate intracellular signaling mechanisms and gene transcription.

Also the cholinergic muscarinic receptors may participate to the control of neurotransmitter release, in particular M1 and M3 presynaptic receptors may stimulate the release of the neurotransmitter as shown, respectively, in the case of dopaminergic [67] and GABAergic [85] terminals in nucleus accumbens; in contrast M2 receptors exert rather an inhibition of neurotransmitter release as shown in the case of the cholinergic terminals in nucleus accumbens [85].

Extensive studies have been conducted *in vitro* and *ex vivo* on the interactions between beta-amyloid and the cholinergic control of neurotransmitter release in various brain areas. Indeed, it has been shown that non neurotoxic beta-amyloid 1-40 concentrations were able to modulate (predominantly, but not exclusively, to inhibit) the release of several neurotransmitters (dopamine, GABA, aspartate, glutamate) elicited by the stimulation of mAChRs and nAChRs subtypes in different brain areas [51, 52, 67, 86-90]. As detailed further below, in the hippocampus, an area which is particularly vulnerable and early target of AD and in which the cholinergic pathways are critical for modulation of attention and memory [49], beta-amyloid regulates the nicotine-evoked release of both excitatory (glutamate and aspartate) and inhibitory (GABA and glycine) aminoacids [90, 91]. The next paragraphs focus in particular on the interference of beta-amyloid on the nicotinic-regulated release of these various neurotransmitters. Although effects were observed also in extrapiramidal (striatum) and limbic (nucleus accumbens) areas (see Tables **1** and **2**) we will focus on hippocampus as the most relevant area, as mentioned, in relation to AD.

Notably, in rat hippocampus both alpha4beta2, and alpha7 nAChRs receptors are expressed [103-105], and it is well known that they have a positive role in regulating cognitive function [106,

Table 1.   Direct interaction between beta-amyloid and nicotinic cholinergic receptors.

Concentration/ time of exposure	Molecular species of Aβ	Aggregation status	Experimental model	Observed effects	Reference
α7 nAChRs					
200pM; 200nM/ 20min	1-42; scrambled 1-42 (control)	Both monomers and oligomers	• Electrophysiological measurements on hippocampal slices from both WT and α7-KO mice treated with the peptide • Behavioral studies on both WT and α7-KO mice previously treated with the peptide by means of ippocampal injection	• 200nM Aβ impaired LTP whereas 200pM Aβ enhanced LTP in slices derived from WT mice • 200pM Aβ enhanced both reference (Morris water maze) and contextual fear memory in WT mice • Aβ effects on LTP and memory were mediated by the activation of α7 nAChRs since they were blocked by α-BgTx (an antagonist of α7 nAChRs) and they were not present in α7-KO mice	[52]
Low nM/up to 7 min	1-42, 42-1(control)	Several oligomeric forms	Measures of Ca^{2+} responses following activation of α7-nAChRs expressed in the axonal varicosities of differentiated hybrid neuroblastoma NG108-15 cells	• Aβ1-42 evoked increases in $[Ca^{2+}]_i$ that were blocked by α-BgTx (an antagonist of α7 nAChRs). The EC50 was between 1-100nM. • Aβ42-1 evoked a slight increase in $[Ca^{2+}]_i$ • Both VGCCs and CICR were involved in the Aβ1-42 evoked increases in presynaptic $[Ca^{2+}]_i$ • Disruption of lipid raft by cholesterol depletion attenuated Aβ1-42 effects	[92]
100nM/ 3-5min	Rat 1-42 and 1-40; human 1-40 and 40-1(control)		Whole-cell patch–clamp recording on rat hippocampal neurons in culture	• Aβ specifically and • reversibly block activation of α7-nAChRs. • This block is noncompetitive, voltage-independent, it does not require the presence of the agonist and is mediated through the N-terminal extracellular domain of the receptor	[93]
100nM for 30 minutes in hippocampal synaptosomes 100nM for 4h in organotypic frontal cortical slices 4.8nmoles/day for 7 days *in vivo*	1-42, (evaluated also 12-28)		• *In vitro* model: hippocampal synaptosomes and organotypic frontal cortical slices • *In vivo* model: chronic i.c.v. injections of the peptide in mice	• In hippocampal synaptosomes Aβ1-42 increased Aβ1-42-α7nAChRs association; this association was reduced by the α7 partial agonist S24795 and Aβ12-28, but not by drugs used for AD treatment: memantine and galantamine. • In hippocampal synaptosomes Aβ1-42 increased tau phosphorylation; this effect was reduced by both α-BTX (an antagonist of α7 nAChRs) and S24795 treatment. • S24795 decreased Aβ1-42 immunostaining after a 4-hrs incubation with the peptide in both organotypic frontal cortical slices and *in vivo* • S24795 reverted Aβ1-42-mediated inhibition of Ca2+ influx through α7nAChRs and NMDA receptors in both organotypic frontal cortical slices and *in vivo*	[94]

(Table 1) Contd….

Concentration/ time of exposure	Molecular species of Aβ	Aggregation status	Experimental model	Observed effects	Reference
100nM fibrillar Aβ for 24h; 10nM and 100nM oligomeric Aβ for 60min	1-40, 1-42, 40-1(control)	Fibrillar and oligomeric Aβ	• *In vitro* model: hippocampal synaptosomes and organotypic frontal cortical slices • *In vivo* model: chronic i.c.v. injections of the peptide in mice • MTT assay in PC12 cells exposed to Aβ alone or to Aβ with α7 nAChR agonists • Receptor binding assays on postmortem brain tissue (superior frontal Gyrus) of 5 AD subjects and 5 controls • Measurement of $[Ca^{2+}]_i$ in SH-SY5Y cells	• 24h incubation with 100nM fibrillar (not oligomeric) Aβ1-40 reduced (65%) cell viability in differentiated PC12 cells. Fibrillar and oligomeric Aβ1-42 reduced cell viability only at μM concentrations • α7 nAChR agonists varenicline (0.1, 1 and 10μM) and JN403 (1μM and 1mM) significantly protect cells against Aβ-induced neurotoxicity (MTT assay) • incubation either with varenicline (1nM and 1μM) or JN403 (1μM) increased [3H]PIB binding in homogenates of frontal cortex tissue derived from the autopsy of 5 AD patients • [125I]Aβ 1-40 bound to α7 nAChRs expressed in both postmortem frontal cortex and in differentiated PC12 cells expressing α7 nAChRs • Oligomeric, but not fibrillar Aβ1-40 (10nM and 100nM), increased [Ca2+]i in SH-SY5Y cells, and this effect was attenuated by varenicline	[95]
500nM/1h before HFS	1-42		Measures of field potentials from hippocampal slices of either rats or α7 KO mice	• Aβ completely inhibited the induction of LTP at 60 min post-HFS • Perfusion of 5μM Nic 10 min before HFS enhanced LTP in both mouse and rat dentate gyrus • Aβ also inhibited the Nic-enhanced LTP but the extent of this inhibition was similar to that in control resulting in LTP remaining • Nicotinic enhancement of LTP in control and in the presence of Aβ was mediated by α7nAChRs since it was absent in α7 null mice	[96]
25nmoles /30-90 minutes	25-35, 31-35		*In vivo* measures of the hippocampal fEPSP before and after i.c.v. injection of the peptides and/or drugs on rats	• Both Aβ25-35 and Aβ 31-35 had no effect on baseline but suppressed HFS-induced LTP • The α-7 selective agonist choline enhanced the Aβ 31-35 induced-suppression of LTP • MLA (an antagonist of α7 nAChRs) partly reversed Aβ 31-35 induced-suppression of LTP	[64]
Both α7 and α4ß2 nAChRs					
300pmoles/day for 14 days	1:1 mixture of Aβ1-40:Aβ1-42, Aβ40-1 (control)		*In vivo* model: chronic i.c.v. infusion (14 days) of the peptides in rats	Chronic Nic treatment prevented Aβ-induced impairments of short-term memory, without affecting memory in normal rats Chronic Nic treatment prevented Aβ-induced alteration of basal synaptic transmission in CA1 area of hippocampus and Aβ-induced inhibition of HFS-evoked LTP Chronic Nic decreased Aβ1-40 levels and reverted the Aβ-induced BACE1 upregulation in the hippocampus Aβ reduced the levels of α7 and α4β2 nAChRs; this effect was reverted by chronic nicotine treatment	[97]

(Table 1) Contd….

Concentration/ time of exposure	Molecular species of Aβ	Aggregation status	Experimental model	Observed effects	Reference
10pM-100nM	1-42, 42-1(control)	Small oligomers	Measures of $[Ca^{2+}]_i$ in hippocampal and cortical synaptosomes from WT mice and mice KO either for the β2 or the α7 subunits	• 100nM Aβ1-42 evoked increases in $[Ca^{2+}]_i$ in hippocampal synaptosomes whereas Aβ42-1 was ineffective. The Aβ1-42-evoked responses were completely blocked by dihydro-β-erythroidine (a nAChRs antagonist with moderate selectivity for the neuronal α4 receptor subunit) and were lost in preparations from β2 KO mice • 10pM-100nM Aβ evoked increases in $[Ca^{2+}]_i$ in cortical synaptosomes. This effect were lost in preparations from α7KO mice and it was abolished by to α-BgTx (an antagonist of α7 nAChRs)	[98]
Non-α7 nAChRs					
1μM for 4-5minutes	1-42		Xenopus Oocyte expressing α4β2 or α2β2 nAChRs	Desformylflustrabromine is a positive allosteric modulator for both α4β2 and α2β2 nAChRs subtypes and it relieved the blockade of these receptors by Aβ1–42	[99]
Undefined nAChRs subtypes					
100nM-2μM	1-42, 12-28, 40-1(control)		Caged-carbachol-induced nicotinic currents from rat hippocampal interneurons measured with whole-cell recordings	• Aβ1–42 and Aβ12-28 reduced nAChR-mediated currents. Aβ40-1 was ineffective • The inhibition of nAChR currents by Aβ1–42 was reversible • Aβ1–42 decreases open channel probability of nAChRs	[100]

Abbreviations: beta-amyloid, Aβ; nAChRs, nicotinic acetylcholine receptors; WT, wild-type, KO, knock-out; LTP, long term potentiation; α-BgTx, α-bungarotoxin; VGCCs, voltage-gated calcium channels; CICR, calcium-induced calcium release; i.c.v., intracerebroventricular; AD, Alzheimer's disease; NMDA, N-methyl D-aspartate; PIB, Pittsburgh Compound-B; HFS, high frequency stimulation; Nic, nicotine; fEPSP, field excitatory post-synaptic potential; MLA, methyllycaconitine; BACE1, beta -site amyloid precursor protein cleaving enzyme 1;

107]. Since the two receptors bind amyloid with different affinity [108], whenever possible, the distinct contribution of the alpa7 and alpha4beta2 nAChRs receptors has been considered.

BETA-AMYLOID/NICOTINE INTERACTION MODULATING INHIBITORY AND EXCITATORY AMINOACID NEUROTRANSMITTER RELEASE IN RAT HIPPOCAMPUS

A brief methodological note may help to better evaluate the reported literature data. When referring to *in vivo* experiments in most cases the neurotransmitter extracellular concentrations (also referred in an abbreviated way as released neurotransmitter) were evaluated by means of brain microdialysis. *In vitro* experiments, if not otherwise indicated, were performed on perfused isolated synaptosomes as originally developed by Raiteri and Raiteri [109]. Isolated perfused synaptosomes have the advantage of providing information only on the direct effects of the *in vitro* added substances because the monolayer perfusion technique prevents indirect interactions due to the release of synaptososmal signalling molecules.

Excitatory Aminoacid Release

Using these approaches it was shown that beta-amyloid 1-40, acutely administered, disorganizes both *in vivo* and *in vitro* the nicotinic control of glutamate and aspartate release in rat hippocampus. In particular, high concentrations of the peptide beta-amyloid 1-40 (respectively 10 microM and 100 nM *in vivo* and *in vitro*) strongly inhibit the release of glutamate and aspartate elicited by nicotine. The observed effect is similar to the one shown in nu-

cleus accumbens and in striatum in the case of dopamine and GABA release following muscarinic cholinergic stimuli [67, 85, 88]. In the *in vivo* experiments also lower concentrations (microM) of beta-amyloid 1-40 inhibited glutamate ad aspartate release. Notably, beta-amyloid 1-40 was unable to inhibit the release of glutamate and aspartate even at high concentrations (up to 10 microM) when the release was elicited using a depolarizing stimulus (veratridine). The lack of effect of beta-amyloid 1-40 against a depolarizing stimulus was observed both *in vivo* and *in vitro*. The lack of inhibitory action of beta-amyloid 1-40 observed in hippocampus with eratridine is siilar to observations in nucleus accumbens and in caudate-putamen when using K+ as depolarizing stimulus [67,88] to promote the overflow of dopamine. It should be noted that these results are at variance with those published using hippocampal slices in which low beta-amyloid concentrations were able to inhibit the release of another neurotransmitter, i.e. acetylcholine [110, 111].

Focusing on the data obtained *in vitro* and *in vivo* in the hippocampus, in the above mentioned experiments beta-amyloid is unable to inhibit the overflow of glutamate and aspartate elicited by depolarization, while decreasing that promoted by nicotinic stimulation is intriguing. One possible explanation is that beta-amyloid 1-40 impairs the nicotine-triggered neurotransmitter release by directly binding to nAChRs or by acting downstream on the cellular signaling machinery. Indeed, hippocampal glutamatergic nerve endings show both alpha7 and alpha4beta2 nAChRs that are capable to controlling neurotransmitter release [112, 113]. Both alpha-7 and alpha-4-beta2 nAChRs may promote aspartate and glutamate

Table 2. **Interactions between beta-amyloid and cholinergic receptors in regulating neurotransmitter release.**

Concentration/time of exposure	Molecular species of beta amyloid[a]	Brain area	Observed effect/putative nicotinic receptor involved	Reference
			***In vivo* effects**	
			Cholinergic nicotinic receptors	
1-10pM, 100nM/20 min	1-42; 12-28; 40-1 (control)	Mouse PFC	Perfusion (microdialysis) of either 100nM Aß1-42 or 100nM Aß12-28 (in the presence of TTX) evoked the release of DA. The Aß1-42-evoked DA release was sensitive to nAChR antagonists, it was absent in α7 KO mice and it was intact in β2 KO mice. Perfusion (microdialysis) of 1-10pM Aß1-42 decreased DA outflow. This decrease in DA release was not significantly affected by nAChR antagonists. Aß40-1 was ineffective	[101]
100nM,1μM,10μM/40-60min	Monomers of 1–40; 40-1 (control)	Hippocampus	Perfusion of different concentrations of Aß (microdialysis): 10μM Aß inhibited the Nic-induced release of GABA, Glu and Asp; 1μM Aß inhibited the Nic-induced release of Glu and Asp;100nM Aß potentiated the Nic-evoked GABA overflow Aß40-1 was ineffective	[90]
10μM/40-60 min	1–40; 40-1 (control)	Hippocampus	Perfusion of 10μM Aß1-40 (microdialysis) reduced the Nic-induced Gly overflow and also the Gly overflow induced by the α7 selective agonist PHA543613 Aß40-1 was ineffective	[91]
			Cholinergic muscarinic receptors	
1-10μM/60-80 min	1-40; 40-1 (control)	Nucleus Accumbens	Aβ (perfused by retrodialysis) is ineffective on both basal and K$^+$-stimulated DA release while it disrupts the muscarinic control of DA release in absence of evident neurotoxicity	[67]
4μM (i.c.v.)/2h or 48h 10μM (retrodialysis)/40 min	1-42	Rat PFC	i.c.v. injection or retrodialysis Aß administration reduced both basal and K$^+$-stimulated DA levels	[102]
100μM/2-3days	LMW oligomers (up to tetramers) of Aß 1-42	PFC	Impairment of the muscarinic regulation of GABAergic transmission (evaluated as IPSCs) in rats injected with the peptide	[68]
			***In vitro* effects**	
			Cholinergic nicotinic receptors	
100pM; 1nM; 100nM/up to 10 min	1–40; 40-1 (control)	Hippocampus	Experiments on isolated nerve endings: 100nM Aß inhibited the Nic-induced release of GABA, Glu, and Asp; 100nM Aß inhibited the release of GABA, Glu, and Asp that was induced by the α7 selective agonist Ch; 100nM Aß inhibited the release of GABA, Glu, and Asp that was induced by the α4ß2 selective agonist 5IA85380; 1nM Aß potentiated the release of Glu induced by Ch; 100pM Aß potentiated the Ch-induced release of both Glu and Asp; Aß40-1 was ineffective	[90]

(Table 2) Contd….

Concentration/time of exposure	Molecular species of beta amyloid[a]	Brain area	Observed effect/putative nicotinic receptor involved	Reference
10nM; 100nM/up to 10 min	1-40; 40-1 (control)	Hippocampus	Experiments on isolated nerve endings: Both 10nM and 100nM Aß inhibited the Nic-induced Gly release; 100nM Aß inhibited the release of Gly evoked by the α7 selective agonist Ch and by the α4ß2 selective agonist 5IA85380	[91]
100nM/up to 10 min	1-40; 1-42	Nucleus Accumbens	Experiments on isolated nerve endings: both Aß 1-40 and 1-42 inhibited the muscarinic control of DA release. Aß 1-40 had a smaller inhibitory effect (about 14%) on the DA release evoked by nicotine	[67]
Cholinergic muscarinic receptors				
10-100nM/up to 12 min	1-40; 1-42; 40-1	Caudate-putamen; Nucleus Accumbens	Experiments on isolated nerve endings: Aß impaired the muscarinic control of DA release in both nucleus accumbens and caudate putamen Aβ affected a specific component of the DA overflow evoked by the non-selective metabotropic glutamate receptors agonist t-ACPD in caudate putamen	[88]
100nM/up to 17 min	1-40; 1-42; 40-1 (control)	Nucleus Accumbens	Experiments on isolated nerve endings: Aß inhibited both GABA and DA release selectively acting on muscarinic receptor subtypes which stimulate transmitter release (M3 and M5) Aß was ineffective on muscarinic receptor subtypes which modulate negatively the stimulated transmitter release (M2 and M4)	[85]

Abbreviations: PFC, prefrontal cortex; TTX, tetradotoxin; DA, dopamine; nAChRs, nicotinic acetylcholine receptors; KO, knock out; Nic, nicotine; Asp, aspartate; GABA, γ-aminobutyric acid; Glu, glutamate; i.c.v., intracerebroventricular; Gly, glycine; LMW, low molecular weight; IPSCs, inhibitory post-synaptic currents; Ch, choline; t-ACPD, trans-1-amino-cyclopentane-1,3-dicarboxylic acid

[a] Aggregation status was directly assessed only in few cases (see for example Mura *et al*. 2012), however concentrations, times of exposure (in particular for *in vitro* experiments) and also delivery method (through a dialysis probe) for the ex vivo experiments do suggest that in most cases the molecular species responsible for the effect is soluble monomeric amyloid.

overflow, however it is not known whether they are located on different nerve endings or act through distinct cellular mechanisms [114]. Further expanding the *in vitro* observations on the effect of beta-amyloid at concentrations equal or greater than 100 nM, it should be noted that the peptide was inhibitory on the release of glutamate and aspartate elicited by both a selective alpha-7 (choline) and alpha-4-beta-2 (5IA85380) agonists. In contrast lower concentrations of the same peptide beta-amyloid 1-40 (1 nM and 100 pM) potentiated the choline induced release of the two excitatory amino acids elicited by the selective stimulation of alpha-7 nAChRs but not that stimulated by the alpha-4-beta-2 agonist (5IA85380). This divergent effect of low versus high *in vitro* beta-amyloid concentrations is interesting considering that physiological concentrations of the peptide range between pM to low nM [115, 116]. These data suggest that perhaps in physiological settings the facilitator effect prevails, while, when the concentrations of the peptide rise, as during pathology, inhibition takes place.

Inhibitory Aminoacids, GABA and Glycine

Both *in vivo* and *in vitro* concentrations of beta-amyloid 1-40, which inhibit glutamate and aspartate release, also inhibit GABA and glycine (see further below) release. The lower *in vivo* concentrations of beta-amyloid, which still inhibited excitatory aminoacids release (1 microM), were unable to affect GABA release.

Notably, *in vivo* 100 nM beta-amyloid 1-40 (the lowest concentration testedin *in vivo* experiments) potentiated the GABA overflow evoked by nicotine. These observations support the concept that beta-amyloid may exert different biological effects when increasing the concentrations, possibly in a continuum from physiology to pathology [51] whit consequent gain and loss of functions. It may be speculated that the potentiation by 100 nM beta-amyloid 1-40 of the nicotine-induced GABA release represents a function which is lost when increasing the concentrations around 1 microM, while the inhibitory effect observed with the 10 microM peptide represents a gain of funtion. These *in vivo* observations were not replicated in *in vitro* experiments. This discrepancy is difficult to explain. However the *in vivo* and *in vitro* results are obtained using concentrations that may differ of more than one order of magnitude. The difference is in part due to the need to use *in vivo* a sufficient amount of the peptide to guarantee the delivery to the tissue through the dialysis probe. It is difficult to compare *in vivo* and *in vitro* results for many reasons. First, *in vivo* concentrations are higher than those used *in vitro* in order It may be speculated that the potentiation by 100 nM beta-amyloid 1-40 of the nicotine-induced GABA release represents a function which is lost when increasing the concentrations around 1 microM, while the inhibitory effect observed with the 10 microM peptide represents a gain of funtion. These *in vivo* observations were not replicated in *in vitro* experiments. This discrepancy is difficult to explain. However the *in vivo*

and *in vitro* results are obtained using concentrations that may differ of more than one order of magnitude. The difference is in part due to the need to use *in vivo* a sufficient amount of the peptide to guarantee the delivery to the tissue through the dialysis probe. Indeed, the delivery to the tissue using this approach is relatively poorly efficient. Only a fraction of the original concentration reaches the brain extracellular compartment. Direct measurements show a positive correlation between the beta-amyloid concentrations delivered through the dialysis probe and the amount of peptide detected by immunostaining techniques in the hippocampal tissue.

On the other hand these experiments lack the precise determination of the concentrations of the peptide within the tissue. Moreover there is no complete information on the kinetics of the delivered peptide, i.e. an evaluation of its accumulation/clearance during the period of infusion and after it.

Experiments based on intraventricular administrations suggest the existence of transport and disposal mechanisms as indicated by an accumulation of the peptide in choroid plexus [102] and amyloid transport at this level has been recently demonstrated [117, 118]. Another difference between the *in vivo* and *in vitro* studies is the timing of exposure to experimental drugs, including beta-amyloid, that ranges in the seconds scale (90 s) *in vitro* and lasts several minutes (typical 40 min) in the *in vivo* studies. In the case of nicotine the latter times may iduce also desensitization of the receptor as suggested by others [119]. Beyond these more technical details, in the *in vivo* experiments there is a substantial difference linked to the fact that the *in vivo* measure of the released neurotransmitters is an integrated function of the multiple interactions between interconnected nerve terminals, i.e. of both direct and indirect mechanisms ultimately controlling the release. In contrast, by methodological definition, when working on supefused synaptosomes only direct effects may be observed. Accordingly, the *in vivo* GABA increase mediated by low concentrations of beta-amyloid could be due to an indirect modulatory role of glutamate and/or aspartate. This interpretation is at least partially supported by the fact that we demonstrated that *in vitro* low beta amyloid concentrations potentiate the release of glutamate and aspartate from synaptosomes, which, if true *in vivo*, might in turn stimulate GABA release through the activation of glutamatergic receptors on GABAergic neurons [120]. Therefore *in vitro* and *in vivo* results cannot be directly compared in all the settings. Finally, the fact that low beta-amyloid concentrations potentiated only *in vivo* GABA release, and only *in vitro* glutamate and aspartate release, while high concentrations were always inhibitory in both conditions, may be due to a differential contribution on the described effects of alpha7 and alpha4beta2 nAChRs. This possibility has been studied *in vitro* by using specific nicotinic agonists.

The cholinergic modulation of glycine release at the presynaptic level on hippocampal nerve endings is modulated both by stimulatory alpha4beta2 and alpha7 nAChRs [121] and by an inhibitory, mAChR subtype [91]. The two modulatory mechanisms display a different sensitivity to beta-amyloid. The stimulatory action of nicotine is inhibited by nanomolar concentration of beta-amyloid 1-40 which was on the contrary inactive on the mAChRs inhibiting glycine release.

This observation allows to speculate that an excess of amyloid may dysregulate the cholinergic modulation of glycine release and in turn hippocampal activity. The decreased release of glycine may have several functional consequences and some of them relevant to the development of AD pathology. Indeed, a decrease of glycine release may lead to a reduction of tonic inhibition [122-124] providing critical neuroprotection under pathological conditions when extracellular glycine levels are elevated [125-128]. A second consequence is the possible decrease of the function of NMDA receptors. Interestingly both the alpha7 and the alpha4beta2 nAChR subtypes, which almost equally contribute to the stimulation of glycine release, are functionally inhibited *in vitro* apparently in a similar

extent by beta-amyloid. The inhibitory effect on both the alpha7 and the alpha4beta2 nAChRs which is partial with a maximal inhibition of about 30-40% may be mediated by the interaction of beta-amyloid with an allosteric binding pocket located within the trans membrane domain of the alpha7 and of non alpha7 nAChRs (see the precedings paragraphs and [129-131]. The *in vivo* observations are paralleled by similar results obtained exposing isolated synaptosomes to the peptide, even if *in vivo* the direct activations of alpha4beta2 receptors was less effective in eliciting glycine release.

The Integrated View

The above reported observations suggest that beta-aamyloid has concentration-dependent effects, potentiating at low, possibly physiological, concentrations the release-promoting effect of nicotinic alpha-7 receptors. In contrast high concentrations of the peptide, possibly pathological, impair the cholinergic responses mediated by both alpha-7 and alpha-4-beta-2 receptors (Fig. **1**). This interpretation is supported by the observation that picomolar beta-amyloid is able to activate alpha-7 receptor currents [119]. Moreover, it has been shown that low picomolar concentrations of beta-amyloid may directly activate alpha-7 receptors and enhance hippocampal LTP and memory [52]. Indeed the peptide seems to physiologically modulate LTP and memory acting upon alpha-7 nAChRs [53]. In line with this observation our data indicate that beta-amyloid in the concentration range between 100 pM and 1 nM may selectively modulate events depending upon alpha-7 receptors, whereas concentrations one order of magnitude higher impair the function of both receptor subtypes. The molecular mechanism mediating such effects of interaction between beta-amyloid and nicotinic receptors has still to be elucidated. Wang and collaborators [108] showed that the peptide can bind both the nicotinic receptor subtypes, but the affinity for alpha-4-beta-2 nAChRs is 100-5000 times lower. Accordingly it is possible that in our experimental conditions the effects of beta-amyloid are due to direct interactions with nAChRs either at the nicotinic binding site [132] or as a consequence of an effect of beta-amyloid on the plasma membrane lipids indirectly affecting receptor activity [133].

The possibility that beta-amyloid may act on cytosolic substrates involved in the synaptic machinery downstream nAChRs cannot be excluded since beta-amyloid can be internalized by cells [134]. We are currently exploring this hypothesis, preliminary data on beta-amyloid synaptosomal entrapping seem however to exclude this possibility. The dual action of low and high bet-amyloid concentrations on alpha-7 mediated neurotransmitter release may rely on various molecular mechanisms including desensitization of these receptors to the activation by beta-amyloid which has been described when using high nanomolar (100 M nd above) concentrations of the peptide [119]. Moreover, increasing the concentrations of beta amyloid -40 in the range 10 pM-1 nM have been reported to activate alpha7 nAChRs, whereas an higher concentration (100 nM) induces desensitization of the receptor [119].Moreover, increasing the concentrations of beta amyloid its may recruit multiple different targets among which synaptosomal proteins undergoing oxidative modifications [135]. Notably, in our experiments we never registered an effect of beta-amyloid on basal neurotransmitter release indicating that all the above mentioned relationships may occur only when circuits are stimulated (as it occurs when hippocampal activity is promoted by learning tasks) and not in resting conditions.

ASSEMBLING THE PUZZLE

Altogether, the reviewed literature strengthens the concept that beta-amyloid is a neuromodulator with effects ranging from synaptic facilitation to inhibition of neurotransmitter release.

The fact that beta-amyloid interacts with the cholinergic control of neurotransmitter release and that increasing the concentrations of the peptide causes the loss of its stimulatory ability and the gain of an inhibitory action are particularly intriguing. The reported data
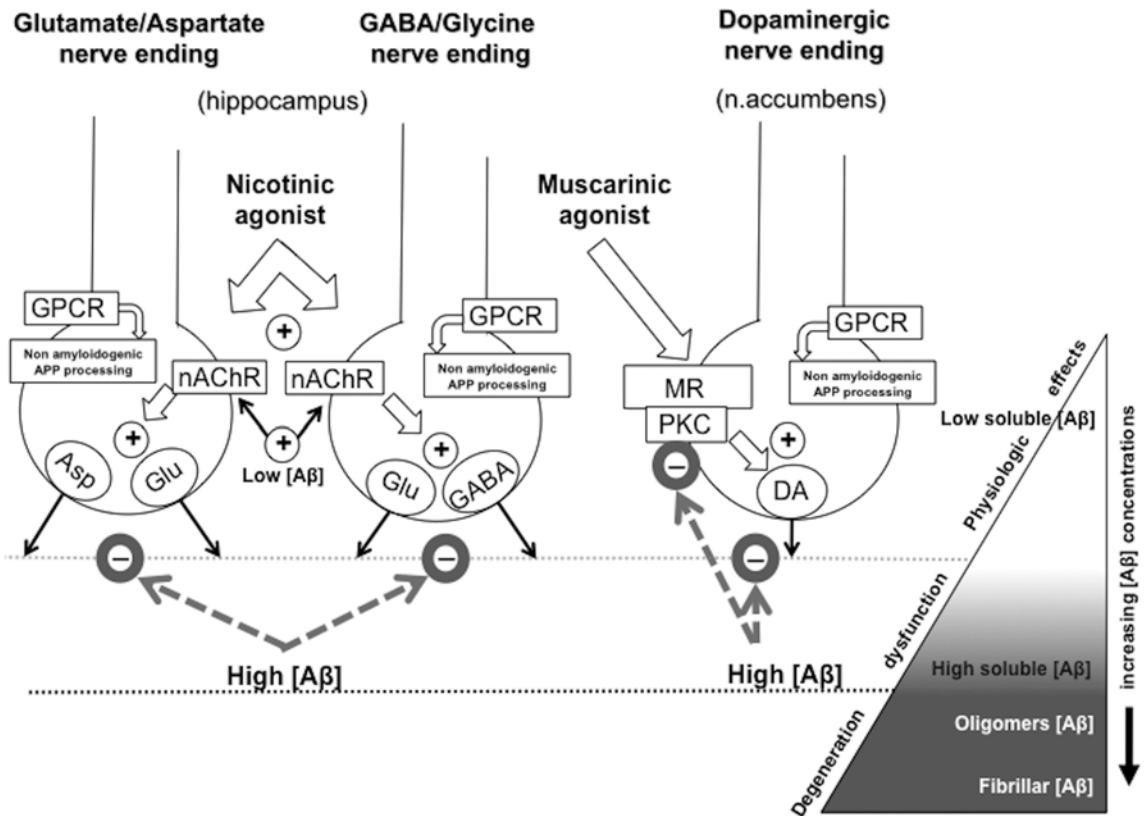
Fig. (1). Putative interactions between cholinergic transmission, APP processing and beta-amyloid

The figure summarizes the observations detailed in the text and in the tables. Beta-amyloid [Aβ] can interact with both nicotinic and muscarinic cholinergic transmission sorting different effects according to the concentration, the cellular model or the brain area investigated in the *ex-vivo* experiments. In particular, low (picomolar to low nanomolar) beta-amyloid concentrations may directly stimulate nicotinic alpha7 receptors and also facilitate the nicotinic-induced release of excitatory (Glu, Asp *in vitro* observations) or inhibitory (GABA, *in vivo* observations) aminoacid transmitters. Increasing beta amyloid concentrations (high nanomolar to low micromolar) may interact with nicotinic alpha4beta2 receptors and desensitize alpha7 receptors. High beta-amyloid concentrations always display an inhibitory effect on the release of several neurotransmitters evoked through the stimulation of nicotinic (nAChRs) and muscarinic receptors (MR) (see text for details). Moreover the stimulation of muscarinic cholinergic and other G Protein Coupled Receptors (GPCR) as well as of nicotinic cholinergic receptors may promote the non-amyloidogenic APP processing, decreasing beta-amyloid formation as well as generating APP products acting as intracellular signals regulating transcription. Beyond a certain point amyloid oligomerizes and then gives origin to fibrils. Oligomers are considered neurotoxic species. Whether beta-amyloid 1-40 or 1-42 are selectively involved is matter of discussion (see text and tables for details). PKC = protein kinase C; DA= dopamine.

indicate also the alpha-7 nicotinic receptor as an important pivot of these events. The whole picture points to the convergence of two main players of Alzheimer's disease pathogenesis, beta-amyloid and cholinergic transmission as part of a mechanism involving early dysfunction of the system rather than degeneration. The reviewed data also suggest that beta-amyloid induced dysfunctions in synaptic transmission may involve different brain transmitters (dopamine, GABA, glutamate, aspartate, glycine) and brain areas (nucleus accumbens, striatum, hippocampus) as well as both the nicotinic and, to a less extent, the muscarinic control of various nerve terminal activities. The emerging picture is that of a multi-transmitter deficit.

It may be speculated that the early derangement of beta-amyloid production may lead to trespass the threshold beyond which beta-amyloid loses the ability to co-promote aspartate and glutamate release, which may be linked to an efficient memory trace formation, and subsequently gains the ability to inhibit the ability of cholinergic stimuli to promote glutamate and aspartate release, impairing at this point memory. These events are eventually followed by neurotoxicity when stably high beta-amyloid concentrations or stably altered 1-40/1-42 ratios lead to the formation of toxic oligomers. Moreover, parallel to this impairment of cholinergic control of glutamate and aspartate release, other effects may be

present in other brain areas involving other neurotransmitters, thus providing the basis for a multi-transmitter deficit in the disease. Moreover individual susceptibility to the failure of the different neurotransmitters (because, for example, of target or metabolism polymorphisms) may in turn be responsible for the various psychiatric symptoms that characterize subsets of patients. These observations suggest the need of redirecting the pharmacological approaches toward multiple neurotransmitter targets in the early stages of the disease and of developing interventions aimed to restore a normal modulator action of beta-amyloid without compromising its putative physiological role.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflicts of interest.

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