Role of the nicotinic acetylcholine receptor in Alzheimer's disease pathology and treatment.

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Abbreviations: Acetylcholine (ACh), Acetylcholinesterase (AChE), Alzheimer's Disease (AD), Amyloid ß peptide (Aß), Amyloid precursor protein (APP), Choline Acetyltransferase (ChAT), desformylflustrabromine (dFBr), familial AD (fAD), Filamin A (FLNA), Knock-out (KO), Methyllycaconitine (MLA), muscarinic acetylcholine receptors (mAChRs), nicotinic acetylcholine receptors (nAChRs), Pregnenolone sulfate (PREGS), positive allosteric modulator (PAM), prefrontal cortex (PFC), Simvastatin (SV).

1. Introduction

Dementia is a debilitating condition frequent in ageing populations, and Alzheimer's Disease (AD) accounts for 70% of all dementia cases. AD is characterized by neuropathological hallmarks consisting of an accumulation of Amyloid ß peptide (Aß) in extracellular plaques, intracellular deposits of tau protein, neuronal loss and, more recently, a prominent synaptic loss was identified (Braak and Braak, 1991; Masliah et al., 2001; Selkoe, 1991; Spires-Jones and Hyman, 2014). In addition, anatomical studies in AD patients showed a massive loss of brain white matter and a specific reduction of cholinergic neurons of the basal forebrain (Auld et al., 2002; Bowen et al., 1976; Coyle et al., 1983; Kim et al., 2013; Whitehouse et al., 1981, 1982). Cholinergic neurons are organized in dense nuclei with widespread projections that entirely cover the central nervous system. In particular, the cholinergic neurons, whose cell bodies are in the basal forebrain, send their long projections to the neocortex and hippocampus (Bigl et al., 1982; Mesulam et al., 1983). Several studies demonstrated the pivotal role of these cholinergic nuclei in cognitive functions. Woolf (1998) proposed a model in which acetylcholine (ACh) release leads to the modulation of cortical circuitry that finally encodes for storage of long-term memory. The cholinergic system is also involved in attention

processes (Muir et al., 1993; Sarter and Bruno, 1997; Wenk, 1997). In a mouse model, the lack of ACh receptors in the prefrontal cortex (PFC) was demonstrated to be responsible for attention deficit, restored by the expression of the receptor in this area (Guillem et al., 2011).

The neurotransmitter ACh binds to two families of receptors, nicotinic acetylcholine receptors (nAChRs) and muscarinic acetylcholine receptors (mAChRs). Both families of receptors regulate the cognitive processes mentioned above (Ghoneim and Mewaldt, 1977; Petersen, 1977; Sarter and Paolone, 2011), and are both affected in AD.

Binding studies performed with the use of [3H]-nicotine and [3H]-ACh showed a significant reduction in nicotine and ACh binding sites in cerebral cortex of patients suffering from AD, demonstrating a decrease of both nAChR and mAChR populations (Gotti et al., 2006a; Paterson and Nordberg, 2000; Perry et al., 1981, 1985, 1987, 1988; Shimohama et al., 1986; Whitehouse et al., 1981, 1982, 1986). In addition to nAChRs and mAChRs, the enzyme choline acetyltransferase (ChAT), involved in ACh production, is also affected in AD. The activity of this ChAT enzyme, and consequently the synthesis of ACh, is decreased in AD brains. In addition, several authors observed a reduction in the activity of acetylcholinesterase (AChE), the enzyme that metabolises ACh after its release in the synaptic cleft (Auld et al., 2002; Bowen et al., 1976; Coyle et al., 1983; Davies and Maloney, 1976; Perry et al., 1978).

The role of the cholinergic system in cognition and the modification observed in neurodegenerative diseases, and in particular in the case of AD, led to the formulation of the "cholinergic hypothesis" of geriatric disorders (Bartus et al., 1982; Contestabile, 2011), according to which the reduction in cholinergic innervation is responsible for the cognitive decline observed in AD patients.

In this context, we will focus on nAChRs, since their involvement in AD has been largely demonstrated, while the contribution of mAChRs has been under-explored. The purpose of this review is to present evidence implicating the role of nAChRs in AD, discuss the data

supporting their interaction with Aß, and the consequences of the perturbation of this interaction in murine models.

2. Brief overview of nAChR subtypes involved in AD

Nicotinic receptors are transmembrane pentameric proteins that belong to the "cys-loop" superfamily of ligand-gated ion channels together with GABAA, GABAC, glycine and 5-hydroxytryptamine (5-HT3) ionotropic receptors (Changeux and Edelstein, 1998; Le Novère and Changeux, 1995). They are composed of a variety of α and β subunits, which determine the pharmacological and kinetic properties of the receptor (Albuquerque et al., 2009; Giniatullin et al., 2005). The five subunits that compose the receptor are assembled around a central hydrophilic pore that mediates the flow of the cations K^+, Na^+ and Ca^{++}. In the human nervous system, there are eight α subunits (α2-α7, α9, α10) and three ß subunits (ß2-ß4) that assemble in different combinations to generate a variety of nAChR subtypes with distinct electrophysiological properties and brain localization (Albuquerque et al., 2009; Gotti et al., 2006b, 2007, 2009).

The use of radioactive ligands allowed nAChR classification into two distinct groups, αBungarotoxin sensitive and αBungarotoxin insensitive receptors (Gotti and Clementi, 2004). Homopentameric α7 nAChRs belong to the first class, while heteropentameric nAChRs containing the ß2 subunit belong to the second class. In this context we will focus on these α7 and ß2 subunits, that were shown to interact with Aß (Liu et al., 2009, 2012; Søderman et al., 2008; Sudweeks and Yakel, 2000; Wang et al., 2000a, 2000b).

The α7 homomeric receptor demonstrates a wide-spread localization in the brain and is characterized by a high calcium ion permeability and a fast desensitization rate (Dani and Bertrand, 2007; Quick and Lester, 2002). α7 nAChR on presynaptic terminals mediate release of others neurotransmitters (Wonnacott et al., 2006), while a postsynaptic or somatic

localization elicits important changes in intracellular Ca^{++} concentration, that can activate second messenger pathways mediating cellular processes such as neuronal survival and gene expression (Berg and Conroy, 2002; Messi et al., 1997; Morley and Happe, 2000). Moreover, it was demonstrated that the activation of α7 nAChRs is important during development for the maturation of glutamatergic synapses (Lozada et al., 2012).

The role of α7 in memory and attention has been investigated for a long time. Knock-out (KO) mice for this subunit did not show a clear cognitive or attention deficit, except when the behavioural paradigm used implied prolonged sessions (Young et al., 2004, 2007). Historically, the first nAChR subunit identified to interact with Aß was α7 (Wang et al., 2000a, 2000b). Later it was shown that Aß is able to activate also ß2*-nAChRs (ß2 subunit-containing nAChRs). This subunit commonly forms heteropentameric receptors in combination with the α4 subunit. The pharmacological and functional characteristics of these heteromeric receptors are determined by both the contributing α and ß subunits. The subtype α4ß2 is characterized by lower calcium ion permeability and a slow desensitization rate compared to the homopentameric α7 nAChR (Quick and Lester, 2002). The "classic" high-affinity nAChR is composed of α4 and ß2 subunits (Zoli et al., 1998). In addition to the α4ß2 subtype, it was demonstrated that the α7 subunit is able to co-assemble with the ß2 subunit to form a heteropentameric receptor. This novel α7ß2 subtype was first investigated by expressing the heteromer in *Xenopus* oocytes. Recently, the α7ß2* nAChR subtype was found in basal forebrain cholinergic neurons and hippocampal interneurons of mouse brain, and in the human basal forebrain (Moretti et al., 2014). This class of receptors seems to be particularly sensitive to Aß-induced toxicity (Khiroug et al., 2002; Liu et al., 2009, 2012). The existence of this novel subtype was further confirmed in a human cell line (SHEP-1) transfected with the cDNA for α7 and ß2 subunits. Under these experimental conditions, α7 and ß2 are able to co-assemble into a functional receptor that localizes at the cell surface. The

ability of α7 and ß2 subunits to form a functional receptor was confirmed in *X. laevis* oocytes. This heteromer displayed only a modest difference in the electrophysiological response to pharmacological agents compared to the homomeric α7 nAChR (Murray et al., 2012). The exact stoichiometry of this recently discovered subtype was not defined. It is clear that functional ligand binding domains could only be formed at the α7-α7 interface. Murray et al. (2012) proposed a schematic model of all the possible stoichiometries for the α7ß2 subtype.

The importance of ß2 in maintaining brain homeostasis during normal ageing was highlighted in the KO mouse for this subunit. Aged ß2 null mutant mice have a thinner cortex compared to age-matched wild-type controls (Zoli et al., 1999). This work should be pursued further as it indicates a "neurotrophic" action of ß2 receptor activation by endogenous ACh (Zanardi et al., 2007). Null mutant ß2 mice were also tested to determine the role of this subunit in cognition. Guillem et al. (2011) showed that these mice exhibit an attention deficit which was restored by re-expression of this subunit with a lentiviral vector in the PFC.

We will now present and discuss the data demonstrating the existence of a physical interaction between nAChR and Aß, the functional consequences of this interaction and the intracellular pathways activated.

3. Interaction between nAChRs and Aß

The interaction between the α7 nAChR and Aß is widely demonstrated. The first indication of this interaction came from the experiments of Wang et al. (2000a, 2000b). They showed that α7 subunits co-localize with $Aß_{1-42}$ in senile plaques of brain slices obtained from patients that suffered from sporadic AD. In this context, no co-localization was found between Aß and the α4 subunit. The strong and specific association between Aß and α7, and no other subunits of the nAChRs, was further demonstrated with immunoprecipitation and Western Blot analysis. This set of experiments showed that $Aß_{1-42}$ is able to immunoprecipitate α7, which was not the

case for other nAChR subunits such as α1, α3, α4, α8 or ß2. The same result was obtained with the reciprocal experiment, α7 immunoprecipitation and $Aß_{1-42}$ detection, meaning that the two proteins strongly interact. Experiments performed with fragments of Aß helped identify the sequence responsible for the interaction with α7, which corresponds to the amino acid residues 12-28 of the Aß sequence (Wang et al., 2000b). Subsequently, competition studies performed by incubating α7 nAChRs with Aß and αBungarotoxin showed that the application of αBungarotoxin is able to decrease the amount of Aß bound to α7 nAChRs, suggesting that both molecules compete for the same ligand binding domain (Wang et al., 2000b). Furthermore, studies using two selective nicotinic receptor ligands, namely [3H]methyllycaconitine and [3H]cytisine, clearly showed that α7 nAChRs bind the less toxic peptide $Aß_{1-40}$, but with lower affinity compared to the 1-42 form (Wang et al., 2000a). Direct interaction between Aß and α7 was further demonstrated in a transgenic mouse line, APPSwe/PS1ΔE9, where α7 was shown to immunoprecipitate with $Aß_{1-40/42}$ (Søderman et al., 2008). Modulation of nAChRs by Aß was also found in *ex vivo* studies: Pettit and colleagues (2001) used rat hippocampal slices to show that $Aß_{1-42}$ incubation is able to reduce postsynaptic currents and open probability of both α7 and non-α7 nAChRs subtypes, demonstrating an interaction between Aß and other nAChR subunits. Even though studies have mainly focused on the interaction between α7 nAChRs and Aß and its functional consequences, it has been demonstrated that also ß2*-nAChRs bind to Aß. The evidence supporting this interaction comes mostly from electrophysiological studies (Lamb et al., 2005; Liu et al., 2009, 2012; Wu et al., 2004).

Several papers from the Yakel laboratory (Lamb et al., 2005; Pandya and Yakel, 2011; Pettit et al., 2001) have investigated the action of Aß on these heteromeric receptors. In both hippocampal interneurons and oocytes they observed a block of ß2*-nAChRs, that could be prevented by the application of a positive allosteric modulator (PAM) specific for ß2 subunit

containing receptors, desformylflustrabromine (dFBr) (Pandya and Yakel, 2011). These are the only conclusive studies to our knowledge, and it will probably be important to continue this dissection.

4. Functional consequences of nAChR-Aß interaction

In this paragraph we will summarize the extensive literature that covers nAChR functional activation or inhibition mediated by Aß, with emphasis on nAChR subtype, Aß concentration and experimental model used. The literature presents conflicting results about functional consequences of Aß binding on nAChRs, eliciting in some studies a receptor activation, and in others an inhibition (Pettit et al., 2001; Puzzo et al., 2008; Tozaki et al., 2002). In order to be able to compare and interpret these contrasting observations, we should be aware of the variability stemming from the different approaches used, such as the difference in receptor subtype (homo- or hetero-pentameric receptors), the system used for electrophysiological recordings (*Xenopus* oocytes that express transiently the subunits versus organotypic slices and cellular systems), and most importantly, the Aß preparation used (concentration and aggregation status). For instance, there is variability in the characteristics and toxicity of the oligomeric vs fibrillar species of Aß (Selkoe, 2011; Walsh and Selkoe, 2004). Below, we will summarize the most remarkable findings over the past years.

Important evidence for a functional interaction between nAChRs and Aß came from the work of Pettit et al (2001). They demonstrated that $Aß_{1-42}$ drives a reversible inhibition of nAChR-mediated currents in hippocampal GABAergic neurons recorded from rat slices. In these experimental conditions the most effective $Aß_{1-42}$ concentration was 500nM, but inhibition was found also at the lower concentration of 100nM. With the use of selective antagonists it was possible to determine that inhibition operates on both α7 and non-α7 receptors (Pettit et al., 2001). Experiments performed on *Xenopus laevis* oocytes transiently transfected with α7

or α4ß2 cDNA showed a concentration dependent effect of Aß on receptor inhibition. In this case the peptide used was $Aß_{1-40}$ and the concentrations adopted were between 0.1 and 10 μM, with increased Aß concentration resulting in a bigger inhibition of the receptor (Tozaki et al., 2002). A different set of experiments demonstrated that Aß enhances ACh activation of the α4ß2 nAChRs expressed in oocytes, this first activation of the receptor was followed by its inhibition (Pym et al., 2005). However, α7 nAChR activation was observed in *X. laevis* oocytes when a range of Aß concentration spanning from 1 to 100 pM was applied (Dineley et al., 2002). Using a different experimental model, Liu et al. (2001) obtained a different outcome. The incubation of cultured rat hippocampal neurons with $Aß_{1-42}$ resulted in inhibition of α7 nAChRs, more precisely of both somato-dendritic and presynaptic populations of receptors. In this case, non-α7 nAChRs were insensitive to Aß inhibition (Liu et al., 2001). Further studies showed an inhibitory effect of $Aß_{1-42}$ on human α4ß2 nAChRs trasnfected in the cell line (SHEP1) (Wu et al., 2004). Other work performed in cellular systems on human nAChRs showed receptor activation. The oligomeric form of $Aß_{1-40}$ was able to activate α7 nAChR expressed in SH-SY5Y cell line (Lilja et al., 2011). Arora et al. (2013) investigated, in a cellular system, the effect of prolonged Aß exposure on nAChR function. The rodent neuroblastoma cell line NG108-15 was transfected with α4ß2 nAChRs and treated for three days with 100 nM Aß. The following acute stimulation with Aß and nicotine led to receptor activation that caused a perturbation of intracellular calcium homeostasis followed by mitochondrial dysfunction and increased oxidative stress (Arora et al., 2013). In a different study, rat hippocampus and cortex were investigated and the activation of both α7 and non-α7 receptors was obtained with an enhancement of Ca^{++} influx into the neuron following the application of picomolar (pM) concentrations of $Aß_{1-42}$. In this case, a distinct response of homomeric and heteromeric receptors was found, specifically, α7 are activated at pM concentrations of Aß, while non-α7 receptors were responsive at nM Aß

concentrations (Dougherty et al., 2003). A study performed on nerve endings with application of Aß demonstrated a non competitive action of $Aß_{1-40}$ on α4ß2 nAChR (Olivero et al., 2014). In conclusion, the outcome of receptor activation or inhibition depends on the system used and on Aß concentration. In general, it is possible to summarize that short incubation and lower concentration lead to receptor activation (Dineley et al., 2002; Dougherty et al., 2003; Puzzo et al., 2008), while longer incubation period and higher peptide concentrations give rise to an inhibitory effect (Dineley et al., 2001; Parri et al., 2011; Pettit et al., 2001). However, due to the variability in the experimental models used, the concentration and aggregation form of Aß (see Table 1), and in the obtained data, it is not possible to definitively conclude whether Aß binding on nAChRs exerts an inhibitory or excitatory effect in a physiological context.

However, it is clear that nAChR-Aß interaction initiates intracellular signalling implicating a set of transduction cascades. Akt phosphorylation mediates the downstream activation of an anti-apoptotic pathway, which is also activated by nicotine treatment (Kihara et al., 2001). The molecular pathways activated are associated with neuroprotection, synaptic plasticity, learning and memory (Plant et al., 2003; Puzzo et al., 2008). Subsequent to incubation with pM concentrations of $Aß_{1-42}$ monomers and oligomers, an increase of hippocampal LTP was observed. This enhancement of synaptic plasticity and the activation of intracellular pathways are mediated by the activation of α7 nAChRs (Dineley et al., 2001; Parri et al., 2011; Plant et al., 2003; Puzzo et al., 2008). An *in vivo* Aß infusion in mice was able to enhance hippocampal dependent memory, highlighted with memory tasks such as the Morris water maze and contextual fear conditioning, which are both hippocampus dependent behavioural tasks (Puzzo et al., 2008). On the other hand, an opposite effect was shown with Aß-nAChR interaction being responsible for inhibition of survival pathways. In this system enhancement of Akt phosphorylation and activation of ERK pathway was observed following α7 agonist

treatment, suggesting that Aß inhibits the neuroprotective effect of α7 nAChR activation (Zhi et al., 2014). Even though some key differences in Aß concentration used among the different studies are present, it is possible to conclude that in certain conditions Aß activates survival pathways. It was then postulated that Aß-nAChR interaction has a physiological role in neuronal homeostasis that is disrupted when Aß concentrations increase in a pathological context, leading to receptor inhibition and possible cellular toxicity (Dineley et al., 2001; Parri et al., 2011).

5. nAChR null-mutants in AD

An interesting approach to study the role of nAChRs in AD *in vivo* is to generate KO transgenic mice for nAChRs subunits expressing fAD (familial AD) associated mutations. This method was used to investigate the role of the α7 subunit in AD, a strategy that has so far not been used for the other nAChR subunits. Two examples from the literature showed strikingly opposite outcomes (Dziewczapolski et al., 2009; Hernandez et al., 2010). In the first study conducted by Dziewczapolski et al. (2009), a mouse model, that we will refer to as APP-α7KO, was generated by crossing an AD model that expresses the human APP (Amyloid precursor protein) with the Swedish (KM670/671NL) and the Indiana (V717F) mutations (Mucke et al., 2000), with the α7 nAChR null-mutant (Orr-Urtreger et al., 1997). This AD model displays spatial memory deficit at 13-16 months of age, while APP-α7KO mice did not exhibit any memory deficit, suggesting that the absence of the α7 subunit of the nicotinic receptor protects against the behavioural deficit caused by expression of the mutated forms of APP in this AD model. It is known that AD mice display some pathological modifications, like loss of synaptic and dendritic markers, such as synaptophysin and MAP2 (Games et al., 1995; Hsia et al., 1999). In the APP-α7KO line the lack of α7 was sufficient to preserve synaptic terminals and dendrites, rescuing levels of synaptophysin and MAP2 to

reach that of aged-matched WT controls. To investigate whether the difference in APP expression in the different mouse lines was responsible of the lack of neuropathology, Western Blot and ELISA analyses were performed. These experiments confirmed that the level of expression of APP and the consequent synthesis of Aß were comparable between mouse lines, demonstrating that the difference in cognitive deficit and neuropathology were mediated exclusively by the nicotinic receptor. In addition, APP mice demonstrated a deficit in LTP while APP-α7KO mice did not display this phenotype. The authors postulated that the absence of α7 could prevent Aß intracellular accumulation ameliorating the cognitive neuropathology and its phenotypic association (Dziewczapolski et al., 2009).

A second study was published by Hernandez et al. (2010). In their work, the transgenic AD model line Tg2576 expressing the human APP sequence with the Swedish mutation (Hsiao et al., 1996) was crossed with the α7 KO mouse (Orr-Urtreger et al., 1997) to establish a different APP-α7KO line. In this series of experiments the mice were tested with a panel of behavioural tasks at five months of age, investigating the role of α7 in early AD. The contextual fear conditioning and the novel object recognition tasks both showed that the cognitive deficits worsen when α7 is absent. In the hippocampus, it was shown that APP-α7KO mice had high levels of Aß, although significantly less than APP mice, an effect which is not due to modification of the APP expression level, equivalent in the two lines. As a consequence of the lower Aß concentration, the plaque load was clearly reduced in APP-α7KO mice. Signs of neuropathology were found in APP-α7KO illustrated by loss of MAP2 immunoreactivity in the hippocampus. In conclusion, this study demonstrated the protective role of α7 nAChRs. The mechanism proposed is that the Aß-α7 nAChR interaction could activate neuroprotective downstream pathways (Parri et al., 2011), and that at the same time the interaction engages Aß preventing its aggregation. With the progression of the disease the amount of Aß increases, it starts to accumulate, and becomes toxic for the neurons

(Hernandez et al., 2010).

In conclusion, α7 nAChRs seem to have a dual effect depending on the age of the mice tested and probably on Aß concentration and aggregation state. However, the AD model used to generate the APP-α7KO line express different fAD associated mutations (Swedish and Indiana mutations in Dziewczapolski et al., 2009; Swedish mutation in Hernandez et al., 2010). The different mutations cause AD pathology to develop in distinct ways, making it difficult to directly compare the results (see Table 2).

6. Pharmacological interference between α7 nAChR-Aß binding

A possible therapeutic approach for AD treatment is the use of nAChR agonists or antagonists to interfere with nAChR-Aß interaction. Numerous molecules with agonist or antagonist activity on nAChRs exist. The protective role of these molecules against Aß toxicity was widely demonstrated using many experimental models and approaches. Gao et al. (2014) investigated the neuroprotective effect of cotinine, nicotine and their analogs, *in vitro* on primary cultures of rat cortical and hippocampal neurons. Cotinine is a nicotine metabolite known for its positive effects on memory and attention and lower toxicity compared to nicotine (Hatsukami et al., 1997). Methyllycaconitine (MLA), an α7 nAChR antagonist, showed neuroprotective effect on mouse and rat primary cell culture (Martin et al., 2004). Another molecule investigated was 2-[2-(4- bromophenyl)-2-oxoethyl]-1-methyl pyridinium (S 24795), a partial α7 nAChR agonist. When this molecule was applied to synaptosomal preparations from rat frontal cortex and post mortem human AD samples it was able to dissociate Aß in a concentration dependent manner. The incubation with S 24795 was able to normalize Ca^{++} influx mediated by both α7 nAChR and NMDAR (Wang et al., 2009, 2010). Many other examples of neuroprotective effects elicited by nAChR agonists exist in the literature. However, here we will focus on the results obtained with *in vivo* experimentation.

The first attempt to treat an AD mouse model with nicotine was performed by Nordberg et al. (2002). The transgenic line harbouring Swedish double mutations (Hsiao et al., 1996) was treated for 5.5 months with nicotine in the drinking water. APP_{Swe} mice showed a reduction in plaque load of 80% compared to sucrose treated mice. The analysis of the Aß fraction reduced by nicotine showed that mainly insoluble $Aß_{1\text{-}40/42}$ was affected while there was no change in soluble Aß (Nordberg et al., 2002). Another study investigated the effect of short and long-term nicotine treatment in the same APP_{Swe} line. Nicotine was administered through subcutaneous injections for 10 consecutive days on 9 month old transgenic mice. For long-term administration, mice were treated with nicotine in the drinking water for a period of 5.5 months. The short-term treatment of 10 days showed a significant reduction in cortical insoluble $Aß_{1\text{-}40/42}$. Long-term nicotine administration elicited a reduction in Aß deposits in blood vessel. APP_{Swe} mice at 14.5 months have fewer αBungarotoxin binding sites, while in transgenic mice treated with nicotine the number of αBungarotoxin binding sites was recovered and comparable to non transgenic age-matched control mice, suggesting that there was an increase in the population of α7 nAChRs (Hellström-Lindahl et al., 2004). Inestrosa et al. (2013) then investigated the effect of intraperitoneal nicotine injections in the APP/PS1 double transgenic line. This line expresses fAD associated mutations in both APP and Presenilin 1 proteins. Mice aged 6 months were treated for one month with nicotine injections, which led to an improvment in working and episodic memory compared to non-treated transgenic mice. This study investigated the effect of nicotine delivery on both young and old mice. For this purpose, 12 month old mice were treated with nicotine injections, and then tested for spatial memory. Like the young mice, they also displayed an improvement in spatial memory, demonstrating that nicotine enhances memory in both young and old mice. The amount of Aß was quantified, and following nicotine injections a reduction in Aß, particularly in the oligomeric form, was found. This work confirmed the observation that AD

mouse models display a reduction of α7 binding sites and PSD-95 puncta reflecting a synaptic deficit in this line. Both α7 binding sites and PSD-95 puncta were increased following nicotine treatment. The explanation proposed by the authors is that α7 nAChR activation through nicotine binding could promote survival pathways and recover the synaptic damage caused by Aß (Inestrosa et al., 2013). Oddo et al. (2005) investigated the consequences of nAChR activation on tau pathology in AD model 3xTg-AD. This line was chosen because it showed cholinergic pathology with a decrease of α7 nAChRs in brain regions affected by Aß deposition and tau aggregation. Chronic nicotine administration was performed in drinking water for 5 months. The long-term nicotine treatment caused faster tau aggregation in CA1 pyramidal neurons. The possible mechanism by which nicotine enhances the aggregation of tau is through the activation of p38-MAP kinase. This kinase is sensitive to Ca^{++}, whose levels are increased following nAChR activation (Oddo et al., 2005). Even though nicotine showed a positive effect reducing plaque load (Hellström-Lindahl et al., 2004; Inestrosa et al., 2013; Nordberg et al., 2002), its use in AD treatment should be limited due to its toxic effect on tau pathology.

Shim et al. (2008) investigated the effect of nicotine administration in an AD mouse model harbouring the Swedish mutation. 12 month old mice were treated with three different doses of nicotine (5 mg/kg, 30 mg/kg and 180 mg/kg) in drinking water for 6 months. Nicotine treatment improved the memory deficit, highlighted with the Morris water maze task. Surprisingly, this study showed a dose dependent increase of α7 nAChR, a result that is in contrast with the literature (Oddo et al., 2005). In the group that received the higher dose of nicotine, the level of α7 nAChR was restored to the level found in wild-type animals (Shim et al., 2008). However, nicotine is addictive and has numerous side effects, for example on the cardiovascular system. The use of cotinine for the treatment of AD was also investigated. We already cited an *in vitro* study on the protective effect of cotinine against Aß toxicity (Gao et

al., 2014). In a mouse model of AD, cotinine treatment decreased the plaque load and was able to activate the Akt pathway, that was shown to be neuroprotective (Echeverria et al., 2011). Cotinine is an α7 nAChR PAM (positive allosteric modulator). It was already demonstrated that this molecule does not display side effects when administrated in humans (Hatsukami et al., 1997). Thus, cotinine is an interesting molecule for AD treatment (for a review see Echeverria and Zeitlin, 2012).

The neuroprotective effect of 4OH-GTS-21, an α7 nAChR agonist, was also investigated. The molecule was administered to PS1 and APP/PS1trangenic mice following a lesion to the fimbria fornix region (FFX). This novel model displayed deficits in spatial memory and reduced neuronal density. Whilst the spatial memory deficit was restored by 4OH-GTS-21 treatment, this molecule had no effect on neuronal density (Ren et al., 2007). Wild-type mice treated with nicotine or with SSR180711, another partial agonist of α7 (Biton et al., 2006), showed increased LTP, while the transgenic AD model APPSwe/PS1ΔE9 showed no effect on LTP following SSR180711 treatment. The authors performed an autoradiographic study to investigate the number of α7 nAChR binding sites. Surprisingly, no decrease was found when transgenic and wild-type mice were compared, a result in contrast with work from other authors (Shim et al., 2008). The molecule SSR180711 seems to be effective in enhancing LTP in wild-type mice but is not able to interfere with Aß-nAChR binding, thus not being considered for AD treatment (Söderman et al., 2011).

Pregnenolone sulfate (PREGS) is an endogenous steroid known to ameliorate cognitive performance in animals. PREGS is a modulator of synaptic plasticity, acting on the activation of glutamatergic transmission (Smith et al., 2014). It was proposed that the positive action of PREGS is mediated by α7 nAChR. In order to investigate this hypothesis, Yang et al. (2012) treated a mouse model obtained with injection of $Aß_{25-35}$ for 7 days whit PREGS. The treatment improved spatial memory and reduced apoptosis in CA1 pyramidal cells. Even

though the protective effect of PREGS is evident, less clear is whether or not it implies α7 nAChR activation (Yang et al., 2012).

Simvastatin (SV) is a statin commonly used in the clinic to control cholesterol levels and it was shown to improve cognitive function in AD patients (Simons et al., 2002; Sparks et al., 2006). In a study conducted by Zhi et al. (2014), this molecule was used to treat a mouse model obtained with injections of the peptide $Aß_{25-35}$. Administration for 11 days of SV improved memory performance in the Morris water maze task and promoted survival of CA1 pyramidal cells. This effect was proposed to be promoted by α7 nAChRs, since it is blocked by MLA administration (Zhi et al., 2014).

A caveat with these pharmacological studies directed at α7 nAChRs is the contribution of potential off-target effects, hence other approaches have been investigated. The intracellular signaling initiated by the binding of Aß to nAChR at the cell surface requires the recruitment of Filamin A (FLNA), a scaffold protein that is known to crosslink actin, and in addition could also have a function in certain intracellular pathways (Stossel et al., 2001). Wang et al. showed that the association between FLNA and the α7 subunit is elevated in AD samples compared to age matched controls. A novel molecule called PTI-125 was used to interfere with the interaction of FLNA and α7. The treatment with PTI-125 prevents FLNA binding to α7 and as consequence reduces the affinity of Aß for nAChRs, attenuating the toxic effect of Aß (Wang et al., 2012).

7. Conclusion

In this review we present evidence supporting the existence of a direct interaction between nAChRs and Aß. The effect of Aß on nAChR physiology is complex. It was indeed shown that this interaction can lead to either activation of survival pathways or to a toxic effect. Under different experimental conditions, Aß can act like an agonist or an antagonist on

AChRs, which underlies the observed variability. Thus, nAChR activation, for example with nicotine, can have a protective effect against Aß exerted toxicity, with the clearance of the peptide. However, this interaction should be studied further to understand the cellular mechanism(s) underlying the activation of survival pathways and Aß clearance, observed following nicotine administration. Moreover, the use of different α7 agonists to target this nAChR subtype is a promising approach that can be translated into AD therapy. Despite the fact that mainly the α7 subunit was investigated in the AD field, some data demonstrating the functional interaction between Aß and non-α nAChRs also exist. We believe that other nAChR subunits could be a target of Aß toxicity and their contribution to AD pathology should be elucidated.

Agonist molecules are responsible for the activation of the receptor at the surface of the cell, and scaffold proteins anchoring nicotinic receptors mediate and regulate the transduction of the intracellular signal. These proteins that interact with the receptors, or directly linked interacting proteins, are largely unknown. In addition to nAChRs, these proteins also present future targets for the study of neurodegeneration research and potential treatment.

Finally, the possibility to generate multiple transgenic models expressing fAD associated mutations and KOs for nAChR subunits was explored. This approach showed apparently contrasting results that however could reflect a different role (protective or not) of the nicotinic receptors depending on Aβ concentration.

Significant evidence suggesting a potential role of nAChRs as therapeutic target in AD exists. These studies provide support for pursuing the investigations to define the detailed mechanism of nAChR neuroprotection and design nAChR agonists, antagonists or allosteric modulators for clinical translation.

Acknowledgments

SL was supported by EU FP7 “BRAINTRAIN”, Fondation pour la Recherche Médicale FRM and Institut Pasteur programme “PasteurInnov” 2012.

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Table 1

Reference	System used	Type of Aβ	Aβ concentration	nAChR subtype	Effect elicited
Petit et al., 2001	Rat Hippocampal slices	$Aβ_{1-42}$	100 nM - 1μM	α7 Non-α7	Inhibition
Tozaki et al., 2002	*X. leavis* oocytes	$Aβ_{1-40}$	0.1-10 μM	α7 - α4β2	Inhibition
Liu et al., 2001	Rat hippocampal neurons	$Aβ_{1-42}$ - $Aβ_{1-40}$	100 nM	α7 α4β2	Inhibition Insensitive to inhibition
Wu et al., 2004	SHEP1 cell line	$Aβ_{1-42}$	1nM	α4β2	Inhibition
Pym et al., 2005	*X. leavis* oocytes	$Aβ_{1-42}$ - $Aβ_{1-40}$	10 nM	α7 α4β2	Inhibition First activation, after inhibition
Dougherty et al., 2003	Rat synaptosomes (hippocampus and cortex)	$Aβ_{1-42}$	100 pM 100 nM	α7 Non-α7	Activation Activation
Dineley et al., 2002	*X. leavis* oocytes	$Aβ_{1-42}$	1-100 pM	α7	Activation
Lilja et al., 2011	SH-SY5Y cells	$Aβ_{1-40}$ (oligomeric)	10^{-8} – 10^{-7} M	α7	Activation
Arora et al., 2013	NG108-15 cells	$Aβ_{1-42}$	100 nM	α4β2	Activation
Oliviero et al., 2014	Rat synaptosomes	$Aβ_{1-40}$	100 nM	α4β2	Inhibition

Table 2

fAD mutations	Phenotype of APP-α7KO mice	Age of mice	Effect of α7	Reference
Swedish (KM670/671NL) Indiana (V717F)	-Rescue of memory deficit -Rescue of synaptic deficit -Rescue of LTP impairment	• 13-16 months • 19-22 months • not specified	Not protective	Dziewczapolski et al., 2009
Swedish (KM670/671NL)	-Memory deficit -Synaptic deficit -Increased plaque load -Decreased Aβ concentration	• 5 months • 5 months • 16-19 months • 5 and 12 months	Protective	Hernandez et al., 2012

Table Legends

Table 1

Action of Aß on nAChRs: The incubation with Aß activates or inhibits nAChRs. Here we summarised the results from the literature with particular attention on the Aß fragment and concentrations used, the model in which the experiments were performed and the nAChR subtype investigated.

Table 2

Conflicting data on α7 KO crossed with AD model: Comparison of two published studies showing the AD model used for the generation of the APP- α7KO line, the phenotype of the obtained APP- α7KO with the age of tested mice and the overall effect of α7 nAChRs.