The α7 nicotinic acetylcholine receptor is highly expressed in hippocampus and in cholinergic projection neurons from the basal forebrain, structures that are particularly vulnerable to the ravages of Alzheimer’s disease. Previous work suggests that β-amyloid peptide can interact with α7 nicotinic acetylcholine receptors, although the nature of this interaction has not been well characterized. To test whether β-amyloid peptide can activate α7 nicotinic acetylcholine receptors, we expressed these receptors in Xenopus oocytes and performed two-electrode voltage clamp recordings, characterizing the response to β-amyloid peptide 1–42 applied at concentrations ranging from 1 pM to 100 nM. In α7-expressing oocytes, β-amyloid peptide 1–42 elicits inward currents at low concentrations (1–100 pM), whereas at higher concentrations (nM), less effective receptor activation is observed, indicative of receptor desensitization. Preincubation with the α7-selective agents, the antagonist methyllycaconitine, and the agonist 4-OH-GTS-21 blocked β-amyloid peptide-induced receptor activation. β-amyloid peptide 1–42 at low concentrations was able to activate the L250T mutant α7 receptor. The endogenous Ca2+-activated chloride current in Xenopus oocytes is recruited upon receptor activation since replacing Ca2+ with Ba2+ in the recording solution reduced current amplitude. Thus, when β-amyloid peptide activation of α7 receptors occurs, these currents are comprised, at least in part, of Ca2+.

Alzheimer’s Disease (AD) is the most common of the senile dementias, the prevalence of which is increasing rapidly with a projected 14 million affected worldwide by 2025. Early on, AD presents clinically as impaired memory formation, yet despite intensive study, the mechanisms underlying AD-related memory dysfunction remain mysterious. Familial AD is associated with several risk factors, the best correlated being age and the inheritance of specific genes (mutations or allele type) that predominately result in increased β-amyloid peptide (Aβ) levels (1, 2, 33).

Although these peptides are present in the brains and cerebrospinal fluid of normal subjects at the picomolar level, substantial evidence indicates that elevated Aβ is a culprit in the cognitive decline of AD (1). Aβ is generated from the amyloid precursor protein through endoproteolytic cleavage by β- and γ-secretases (2). In normal individuals, Aβ(1–40) (Aβ40) comprises the majority of the Aβ population; a far smaller fraction is made up of Aβ42 (1). Aβ42 is highly fibrillogenic and exhibits toxic and toxic effects on neurons (3–5). The hippocampus is a locus for the earliest detected cognitive dysfunction in AD: impairment in the encoding of new episodic memories is typical of the earliest stages of AD, and the loss of episodic memory in AD is linked to medial temporal pathology inclusive of the hippocampus (6–8). Despite intensive study, the mechanism by which elevated Aβ leads to AD-related hippocampal dysfunction remains mysterious, not to mention the lack of an understanding of the normal physiologic role for Aβ in synaptic function and signal transduction.

As yet, a receptor for Aβ that is capable of influencing synaptic plasticity in the hippocampus remains unidentified. Cholinergic connections between the hippocampus and cortical structures within the temporal lobe and the cortical cholinergic system that originates within the basal forebrain are selectively vulnerable in the course of AD (9–11). The loss of proper functioning of these neuronal populations is thought to underlie the loss of memory in AD patients (12, 13). The α7 nicotinic acetylcholine receptor (nAChR) is highly expressed on neurons of hippocampus and cholinergic projection neurons from the basal forebrain. A number of recent studies have convincingly demonstrated an interaction between the α7 nAChR and Aβ in vitro and on neurons. For instance, it has been shown that Aβ42 co-immunoprecipitates with the α7 nAChR in samples from postmortem AD hippocampus and that α7 nAChR antagonists compete for Aβ42 binding to heterologously expressed α7 nAChRs (14). Furthermore, preincubation with Aβ42 antagonizes the activation of α7 nAChR-like currents in hippocampal neurons, and Aβ42, acting through α7 nAChRs, can elicit extracellular signal-regulated kinase (ERK) MAPK activation in hippocampal cultures (15, 16). This last observation is likely triggered by Ca2+ influx; α7 nAChRs are highly permeable to this pluripotent second messenger (17).

We tested the hypothesis that Aβ could directly activate the α7 nAChR by expressing these receptors in Xenopus oocytes and performing two-electrode voltage clamp recordings following perfusion with pm to nm concentrations of non-aggregate Aβ. Both Aβ40 and Aβ42 were capable of eliciting inward currents from α7 nAChR-expressing oocytes; however, the current amplitudes resulting from Aβ42 receptor activation were much larger than those generated by Aβ40. Inward currents elicited by Aβ42 were comprised of Ca2+, were blocked by the α7 nAChR-selective antagonist methyllycaconitine (MLA), and were cross-desensitized by the α7 nAChR-selective agent 4OH-GTS-21. We obtained evidence that low concentrations of Aβ42 are highly desensitizing; wash times up to 30 min were unsuc-
cessful at allowing subsequent Aβ42 activation. Desensitization induced by low concentrations of Aβ42 did not have an observable effect on subsequent nicotine stimulation. However, prolonged exposure to a high concentration of Aβ42 led to cross-desensitization of nicotine responses, suggesting the existence of more than one Aβ42 binding site or an Aβ42-induced conformation that differentially interferes with nicotine activation of α7 nAChRs. Overall, we conclude that Aβ42 is a high affinity ligand for α7 nAChRs that is capable of gating Ca\(^{2+}\) flux through the channel.

**EXPERIMENTAL PROCEDURES**

**Oocyte Expression—Xenopus leavis** (Nasco) oocytes were harvested and prepared for injection as described (17). Rat α7 nAChR cDNA or rat α7 nAChR cDNA containing the point mutation L250T, both contained in the cytomegalovirus expression vector pcDNA3/Amp (Invitrogen), were utilized for electrophysiology. α7 nAChR cDNAs were obtained from the laboratory of Dr. Jim Patrick.

Stage VI oocytes were injected intracellularly with 10–20 ng of cDNA 1 day following harvest using a Drummond nanoinjector. Oocytes were maintained at 17 °C in SOS medium (100 mM NaCl, 2 mM KCl, 1.8 mM CaCl\(_2\), 1 mM MgCl\(_2\), 0.1% bovine serum albumin, and 25 μg/ml gentamicin) prior to recording.

**Electrophysiological Recordings—**Membrane currents were recorded from oocytes 3–7 days post-injection. Oocytes were bathed at a rate of 10 ml/min in the recording chamber with oocyte Ringer’s solution (115 mM NaCl, 2.5 mM KCl, 1.8 mM CaCl\(_2\), 10 mM HEPES, pH 7.2) except for experiments using Ca\(^{2+}\)-free Ringer’s solution in which 1.8 mM BaCl\(_2\) was substituted. Drugs and peptides used were: MLA (Sigma), nicotine tartrate (Sigma), 3-(4-hydroxy-2-methoxybenzylidene)anabaseine (4-OH-GTS-21 (18)) generously provided by Dr. Roger Papke, University of Florida), human Aβ(1–40) and the reverse peptide (40–1, Sigma), rat Aβ(1–42) (Calbiochem). 100 μM stocks of Aβ were prepared to promote solubility and to retard fibril formation, as described (19); all compounds were diluted in Ringer’s solution prior to electrophysiological recordings.

Two-electrode voltage clamp was performed at ambient temperature utilizing an Axoclamp-2A amplifier. Current and voltage pipettes were filled with 3 M KC1, and resistances ranged from 0.1 to 0.5 megohms. Oocytes were voltage-clamped at −60 mV unless otherwise stated. Oocytes with resting membrane potentials between −20 and −60 mV and voltage clamp injections of less than 100 nA were used. Solution exchange was achieved by using electromagnetic valves (type 1; General Valve Corp.). Data were collected at a rate of 1.667 and 5 kHz for 30- and 5-s drug applications, respectively. Data acquisition and analysis was performed with pCLAMP software (Axon Instruments, Inc.). Current amplitudes were determined by measuring the maximum negative deflection from baseline. Data normalized and reported as percent current of equivalent magnitude to the test perfusion with nicotine. Scale bars = 500 nA × 1 s.

**RESULTS**

Our work utilized rat Aβ42 peptide that was prepared under conditions to retard aggregation and fibrillization (21, 22). We tested our Aβ42 preparations for aggregation in a Congo red assay. At the concentrations used and under the incubation conditions tested, none of the Aβ42 preparations significantly pelleted out of solution with Congo red dye at 2.5 or 25 μM (data not shown (23)).

**Aβ Activates α7 nAChRs**—The ability of low concentrations of non-fibrillar Aβ42 to activate α7 nAChRs was tested by expressing these receptors in the Xenopus oocyte expression system and performing two-electrode voltage clamp recordings. Oocytes positive for α7 nAChR expression, as determined with a 5-s perfusion of 100 μM nicotine, were washed for 5 min with Ringer’s solution and then subjected to a 5-s perfusion with 10 μM Aβ42. This concentration of Aβ42 led to inward currents that averaged 44 ± 12 nA (−60-mV holding potential, n = 8) or 176 ± 76 nA (−100-mV holding potential, n = 4) in amplitude (Fig. 1a). Prior activation with nicotine was not necessary to observe Aβ42-induced responses. We observed receptor activation following Aβ42 perfusion on oocytes that had not yet been exposed to any other α7 nAChR agonist. Furthermore, we did not observe measurable current responses when mock-injected oocytes were subjected to Aβ42 perfusion (n = 12, data not shown). We also tested whether the far more abundant form of amyloid peptide, Aβ40, was capable of activating α7 nAChRs expressed by Xenopus oocytes. 10 pM human Aβ40 generated inward currents from α7 nAChR-expressing oocytes. However, the peak current amplitudes were much smaller than an equivalent concentration of Aβ42; the normalized Aβ40 currents were less than 20% of those generated with Aβ42. 10 pM Aβ40 elicited 10.13 ± 1.88 nA peak current, which is 1.75 ± 0.64% of the 100 μM nicotine response (n = 8, data not shown). Finally, the reverse (human 40–1) peptide failed to induce α7 nAChR activation (n = 10, data not shown). These experiments demonstrate that Aβ42, as well as Aβ40, is a selective and high affinity agonist for α7 nAChRs expressed in oocytes.
Next, we evaluated whether we could generate \( \alpha 7 \) nAChR activation with repeated application of \( \alpha B 42 \). Repeated activation of \( \alpha 7 \) nAChRs with nicotine could be achieved by performing 5-min washes between drug perfusions. In contrast, following a 5-s perfusion with 10 pm \( \alpha B 42 \) that yielded a current response, we were unable to measure a second \( \alpha B 42 \) response (Fig. 1b). This was the case for wash times of up to 30 min. Interestingly, at this low concentration of \( \alpha B 42 \), loss of the \( \alpha B \) response did not interfere with the nicotine response. Perfusion with 10 \( \mu M \) nicotine within 1 min of perfusion with the second application of \( \alpha B 42 \) resulted in a nicotine response that was indistinguishable from the nicotine test application (\( n = 5; \) Fig. 1b). Clearly, the activation properties of low concentrations of \( \alpha B 42 \) and of 100 \( \mu M \) nicotine on \( \alpha 7 \) nAChRs are different.

High Concentration of \( \alpha B 42 \) Cross-desensitizes \( \alpha 7 \) nAChRs—Previous reports have demonstrated \( \alpha B 42 \) antagonism of \( \alpha 7 \) nAChRs expressed by neurons (15, 16). These studies utilized high concentrations (100 nm-1 \( \mu M \)) of \( \alpha B 42 \) and tested \( \alpha 7 \) nAChR responses following preincubation with the peptide. We therefore attempted to cross-desensitize the nicotine response of \( \alpha 7 \) nAChRs expressed by oocytes with 100 nm \( \alpha B 42 \). A 2-min preincubation (co-application of nicotine and \( \alpha B 42 \)) did not antagonize the receptor response with 100 nm \( \alpha B 42 \) inhibited the responses of the receptors to subsequent 100 \( \mu M \) nicotine application (53.2 \( \pm 4.9\% ; \) \( n = 11; \) Fig. 1c). These results are consistent with the published reports that 100 nm and 1 \( \mu M \) \( \alpha B 42 \) antagonize ~60–80\% of neuronal \( \alpha 7 \) nAChR responses to agonist (15, 16).

\( \alpha 7 \) nAChR-specific Agents Block Receptor Activation by \( \alpha B 42 \)—We next demonstrated that the \( \alpha B 42 \) effect could be blocked by the \( \alpha 7 \) nAChR-selective antagonist, MLA. Fig. 2a shows a representative experiment from nine replications in which MLA blocked both the nicotine and 10 pm \( \alpha B 42 \) response from an oocyte expressing \( \alpha 7 \) nAChRs. Recovery of the nicotine response follows a 10-min washout with Ringer’s solution (\( n = 13; \) Fig. 2b). Following positive responses to first nicotine and then to 4-OH-GTS-21, a 5-min perfusion with 4-OH-GTS-21 prevented subsequent activation of \( \alpha 7 \) nAChRs with 10 pm \( \alpha B 42 \). A 10-min washout with Ringer’s solution restored the \( \alpha B 42 \)-induced current. 4-OH-GTS-21 cross-desensitized the nicotine responses as well (data not shown). The fact that a response to a second application of \( \alpha B 42 \) was observed after washout suggests that 4-OH-GTS-21 binding prevents \( \alpha B 42 \) binding and that the two binding sites overlap. These experiments further demonstrate that \( \alpha B 42 \) is a selective agonist for \( \alpha 7 \) nAChRs expressed in oocytes.

\( \alpha B 42 \) Activates the L250T \( \alpha 7 \) nAChR—We utilized the desensitization-resistant mutant L250T \( \alpha 7 \) nAChR to exploit the large amplitude currents generated by this receptor type (30, 31). Following a test application of 1 \( \mu M \) nicotine and a 5-min wash with Ringer’s solution, 10 pm \( \alpha B 42 \) was perfused for 30 s, which resulted in a substantial inward current (1606 \( \pm 280\) nA, \( n = 28; \) Fig. 3a). Shorter drug application times (5 s) yielded similarly shaped current responses (Fig. 3b). These data indicate that, as is true of the wild-type receptor, the L250T mutant version of the \( \alpha 7 \) nAChR binds and is activated by \( \alpha B 42 \). Given the marked difference in the shape of the nicotine-induced currents versus the \( \alpha B 42 \)-induced currents, the data also suggest that \( \alpha B \) is more effective at eliciting desensitization of the L250T mutant receptors than is nicotine.

Unlike wild-type \( \alpha 7 \) nAChRs, we were able to generate repeated responses to 10 pm \( \alpha B 42 \) from the L250T mutant receptor following a 10-min wash between \( \alpha B \) applications (Fig. 3b).
tration increases, the number of oocytes that fail to respond to 
Aβ42 increases although they proved positive for α7 nAChR 
expression (Fig. 4c). In other words, with increasing Aβ42 
concentration, a larger proportion of oocytes responsive to 
nicotine fail to respond to Aβ42 perfusion.

We also evaluated various concentrations of human Aβ40 for 
its ability to activate the L250T α7 nAChR. As was observed for 
wild-type α7 nAChRs, Aβ40 activated L250T receptors to a 
much lesser extent than an equivalent concentration of Aβ42. 
Table II lists peak current amplitudes for 10 pm, 1 nM, and 100 
nM Aβ40 application to L250T α7 nAChRs expressed in oocytes. 
Crude analysis indicates that Aβ40 is less efficacious than 
Aβ42 at receptor activation.

*Aβ42 Activation of α7 nAChRs Leads to Ca2+ Influx—Xenopus 
oocytes endogenously express a Ca2+-activated chloride 
channel that produces a net inward current when activated. α7 
nAChRs are highly permeable to Ca2+ (17) such that when 
expressed in Xenopus oocytes, receptor activation leads to 
avtivation of the endogenous Ca2+-activated chloride channel. 
Replacement of Ca2+ ions with Ba2+ ions in the Ringer’s solution 
greatly reduces α7-nAChR generated currents because the 
outward chloride current no longer contributes to the whole cell 
current. We tested the possibility that Aβ42 activation of α7 
nAChRs leads to Ca2+ influx by performing recordings of the 
L250T mutant α7 nAChR following agonist applications in the 
presence and absence of Ca2+ in the perfusion buffer.

Fig. 4d illustrates that, in the presence of Ca2+, L250T α7 
nAChRs exhibit large inward currents in response to both 
nicotine and Aβ42. In the absence of Ca2+, L250T α7 nAChRs 
have reduced nicotine and Aβ42 responses, yet the current 
amplitudes are still rather large. In Ca2+-free Ringer’s solution, 
the nicotine and Aβ42 responses were 44.65 ± 8.30% and 
43.62 ± 9.06%, respectively, of those in Ca2+ Ringer’s solution 
(n = 7). These results suggest that: 1) currents induced by 
either nicotine or Aβ42 are significantly contributed to by the 
Ca2+-activated chloride channel; 2) Ca2+ influx occurs with 
both nicotine and Aβ42 activation of α7 nAChRs; and 3) in the 
absence of Ca2+, ion flux still occurs through these receptors.

**Discussion**

We have demonstrated that rat Aβ42 peptide prepared in 
non-aggregate form directly activates rat α7 nAChRs expressed 
by Xenopus oocytes. This is a high affinity interaction; 
concentrations as low as 100 fm Aβ42 are capable of inducing inward 
currents. Aβ42 is competitively antagonized by MLA and cross-
desensitized by 4-OH-GTS-21, both α7 nAChR-selective 
agents.

On wild-type receptors, the range of effective Aβ42 doses 
extends over the amount of soluble Aβ that occurs in normal (pm) 
as well as in AD (pm–nm) brain (1). This suggests the 
possibility of an Aβ-α7 nAChR interaction under normal phys-
ologic conditions, and thus we propose that Aβ may be an 
endogenous ligand for α7 nAChRs. Furthermore, our data sug-
gest that part of the pathology elicited by Aβ in AD may be due 
to aberrant activation of α7 nAChRs. In particular, as the α7 
nAChR is a ligand-gated ion channel highly permeable to Ca2+ 
(17), chronic activation of α7 nAChRs in AD could lead to 
dysregulation of Ca2+ homeostasis and provide a molecular 
mechanism for the cholinergic dysfunction that is a hallmark of 
AD (9–11).

The more prevalent form of amyloid peptide in brain, Aβ40, 
was also able to activate α7 nAChRs. However, Aβ40 was less 
effective than an equivalent concentration of Aβ42 at activat-
ing the wild-type and L250T mutant α7 nAChRs. It should be 
noted, however, that human and rat Aβ sequences differ by 
three amino acids; the efficacy of human Aβ40 might be dimin-
ished relative to rat Aβ40 on rat α7 nAChRs. Whether there is 
a marked difference in the efficacy of rat versus human Aβ 
peptides at α7 nAChRs is unknown at this time. Nonetheless, 
our data suggest that Aβ40, as well as Aβ42, is capable of 
avtivating α7 nAChRs in situ.

One model we have proposed previously and that is consist-
ent with our current findings is that hippocampus-dependent 
learning and memory impairments in early AD arise in part 
because of the increased Aβ burden and chronic activation of 
the ERK MAPK cascade in the hippocampus through α7 
nAChRs (19). In support of this, we have demonstrated that 
elevation of Aβ in vivo using an animal model for AD (Tg2576 
(24)) leads to the up-regulation of hippocampal α7 nAChR 
protein (19). α7 nAChR up-regulation in the hippocampus 
of Tg2576 animals is coincident with the manifestation of a con-
textual fear learning deficit (34), a hippocampus-dependent 
associative learning paradigm (25, 26). Furthermore, increased 
α7 nAChR protein levels are detected concomitantly with dys-
regulation of the 42-kDa isoform of ERK MAPK (19). Consid-
ering that ERK MAPK activity is necessary for rodent fear 
learning, α7 nAChR up-regulation in hippocampus may serve 
as a biochemical marker for the synaptic plasticity impair-
ments and learning and memory deficits in Tg2576 animals 
that result from chronic elevated Aβ (27–29).

Our observation of sustained inactivation of α7 nAChRs by 
low concentrations of Aβ42 may reflect prolonged occupancy of 
its binding site(s) on α7 nAChRs. This putative long-lasting 
interaction is consistent with the hypothesis proposed by 
Wang et al. (32) that α7 nAChRs may seed or nucleate Aβ depo-
sition, eventually leading to plaque formation. On the other 
hand, if the conformation of Aβ in the receptor-bound state is incompa-
tible with further aggregation, α7 nAChRs may decrease 
the amount of free Aβ, thus slowing Aβ aggregation until levels of 
Aβ rise beyond the binding capacity of α7 nAChRs.

L250T mutant α7 nAChRs were utilized to confirm our ob-
servations of Aβ42 effects on wild-type receptors because these 
mutant α7 receptors generate much larger, longer lasting cur-
rent responses (30, 31). These studies again demonstrated a 
high affinity interaction of Aβ42 with the α7 nAChR. Consist-
Aβ42 activates α7 nAChRs in a concentration dependent manner; receptor activation leads to Ca2+ influx. Wild-type (a) and L250T mutant α7 nAChR (b) concentration response relationships reported as a percentage of the nicotine (nic) response to 100 and 10 μM nicotine, respectively. Holding potential, −60 mV. Average percentage of nicotine response for wild type: 1 pm = 4.95 ± 1.93; 10 pm = 10.01 ± 2.4; 100 pm = 12.11 ± 2.12; 1 nm = 4.93 ± 1.57; 100 nm = 1.50 ± 0.91. Average percentage of nicotine response for L250T: 1 pm = 17.10 ± 8.73; 10 pm = 47.56 ± 5.74; 100 pm = 86.40 ± 5.32; 100 nm = 24.87 ± 4.52. Table I displays the average peak current amplitudes for each concentration tested. The fraction of oocytes that responded to nicotine but failed to respond to Aβ42 increases as the concentration of Aβ42 increases (c). Replacement of Ca2+ ions in the Ringer’s solution with Ba2+ reduces, but does not eliminate, the currents induced by either nicotine or Aβ42 (d). Average peak current values are as follows: nicotine in Ca2+ Ringer’s solution = 2759 ± 319 nA; nicotine in Ba2+ Ringer’s solution = 1232 ± 229 nA; Aβ42 in Ca2+ Ringer’s solution = 1806 ± 275 nA; Aβ42 in Ba2+ Ringer’s solution = 700 ± 146 nA.

Table I
Average peak current amplitudes resulting from different Aβ42 concentrations applied to wild-type or L250T mutant α7 nAChRs expressed in oocytes

| Aβ42 concentration | WT Current amplitude | L250T Current amplitude |
|--------------------|----------------------|-------------------------|
| 0.0001             | 16.95 nA             | 6.36 nA                 |
| 0.001              | 29.24 nA             | 4.95 nA                 |
| 0.01               | 53.94 nA             | 21.47 nA                |
| 0.1                | 52.91 nA             | 21.47 nA                |
| 1.0                | 14.83 nA             | 6.36 nA                 |
| 100                | 5.90 nA              | 24.87 nA                |

Table II
Average peak current amplitudes resulting from different Aβ40 concentrations applied to wild-type or L250T mutant α7 nAChRs expressed in oocytes

| Aβ40 concentration | Current amplitude |
|--------------------|-------------------|
| 0.01               | 255.13 nA         |
| 1.00               | 194.71 nA         |
| 100                | 41.55 nA          |

Aβ activates α7 nAChRs through α7 nAChRs in hippocampal slices (19). This activation requires extracellular Ca2+ and does not require action potential propagation. Thus, it is conceivable that ERK MAPK activation in hippocampus results from Ca2+ influx directly through α7 nAChRs following Aβ-activation.

One notable feature of the L250T α7 nAChR current response to nicotine versus Aβ42 is the current profile. Whereas nicotine and other agonists for this receptor induce little desensitization during drug perfusion (30, 31), at all concentrations of Aβ42 tested, Aβ application leads to more rapid closure of L250T channels than 1 or 10 μM nicotine. This observation may reflect desensitization or open channel block. We favor the interpretation that Aβ42 induces desensitization rather than acting as an open channel blocker since during washout, we do not observe a rebound outward current as a result of channel de-block. Furthermore, the ability to fully and rapidly activate α7 nAChRs with nicotine following inactivation of the Aβ42 response is consistent with an unblocked channel. Thus, Aβ42 appears to induce L250T α7 nAChR channel closing in a different manner than previously tested agents.
Aβ42 is highly desensitizing to wild-type receptors in that high concentration or repeated application of Aβ42 inactivates the Aβ response in wild-type receptors. However, inactivation of the Aβ response with low concentrations of Aβ42 (10 pM) does not interfere with subsequent nicotine responses. Consistent with our data, Liu et al. (16) did not observe significant inhibition of α7 nAChR responses to acetylcholine on cultured hippocampal neurons following preincubation with Aβ42 at concentrations below 1 nM. These observations imply that the high affinity (low pM) Aβ42 binding site and the nicotine binding site do not overlap or that the conformation induced by low concentrations of Aβ42 interferes with subsequent Aβ activation but not with nicotine binding or receptor activation.

We were able to inhibit nicotine activation of α7 nAChRs expressed in oocytes by preincubation with 100 nM Aβ42. These findings are in agreement with published reports by Petit et al. (15) and Liu et al. (16) that preincubation with compounds targeted to blocking the effects of Aβ42 antagonized subsequent activation of neuronally expressed α7 nAChRs. These observations suggest that a second, lower affinity binding site exists for Aβ42 that overlaps with the “traditional” agonist binding site. Alternatively, extended exposure to high concentrations of Aβ42 may induce a conformational change in the receptor that is incompatible with subsequent activation with traditional agonists. The presence of a second, lower affinity site for Aβ42 binding is supported by studies performed by Wang et al. (32) in which two K values were detected with [3H]MLA competition binding. K values for Aβ42 binding to rat α7 nAChRs prepared from cortex and hippocampus were 4.1 and 440 pM, respectively. Furthermore, these binding constants are compatible with our observation that 1 pM Aβ42 activates α7 nAChRs.

The work presented here suggests that Aβ42/α7 nAChR interactions may play a role in the etiology of AD. We have demonstrated that the Aβ peptide functions as a ligand for α7 nAChRs, and we provide evidence that the binding and activation properties of Aβ42 are somewhat distinct from those of agonists such as nicotine. Specifically, low concentrations of Aβ42 activate the receptor and desensitize it to further activation by the peptide but not by nicotine; higher concentrations inhibit peptide activity and cross-desensitize it to nicotine. The unusual nature of Aβ42-induced receptor activation and desensitization indicates that the in situ effects on α7 nAChRs could prove to be quite complex. In general, however, our data indicate that compounds targeted to blocking the effects of Aβ on α7 nAChR function may be a promising therapy for AD.

Acknowledgments—We thank Dr. Laura Schrader and Amber Mayne for troubleshooting expertise with oocyte preparation and electrophysiology. We thank Dr. Jim Patrick for insightful advice throughout the execution of this project. We are grateful to Dr. Roger Papke for providing 4-OH-GTS-21 and for helpful comments during manuscript preparation.

REFERENCES

1. Kuo, Y. M., Emmerling, M. R., Vigo-Pelfrey, C., Kasunic, T. C., Kirkpatrick, J. B., Murdoch, G. H., Ball, M. J., and Roher, A. E. (1996) J. Biol. Chem. 271, 4077–4081
2. Selkoe, D. J. (1998) Trends Cell Biol. 8, 447–453
3. Lambert, M. P., Barlow, A. K., Chrony, B. A., Edwards, C., Freed, R., Lissatos, M., Morgan, T. E., Rozovskaya, I., Tremor, B., Viola, K. L., Wals, P., Zhang, C., Finch, C., Graff, G. A., and Klein, W. L. (1998) Proc. Natl. Acad. Sci. U. S. A. 95, 6444–6453
4. Hartley, D. M., Walsh, D. M., Ye, C. P., Dhill, T., Vasquez, S., Vassilev, P. M., Teplow, D. B., and Selkoe, D. J. (1999) J. Neurosci. 19, 8876–8884
5. DeFrancesco, J. C., Mathis, C., and Changeux, J. P. (2000) Rev. Neurol. 11, 75–83
6. Butters, N., Granholm, E., Salomon, D. P., Grant, I., and Wolfe, J. (1987) J. Clin. Exp. Neuropsychol. 9, 479–497
7. Fox, N. C., Warrington, E. K., Freeborough, P. A., Hartikainen, P., Kennedy, A. M., Stevens, J. M., and Zisser, M. N. (1998) Brain 119, 2001–2007
8. Fox, N. C., Warrington, E. K., Stevens, J. M., and Rossor, M. N. (1996) Ann. N. Y. Acad. Sci. 777, 226–232
9. Brown, D. M., Smith, B. C., White, P., and Davison, A. N. (1976) Brain 99, 459–498
10. Davies, P., and Maloney, A. J. (1976) Lancet 2, 1403
11. Perry, E., Perrin, R. B., Bledsoe, G., and Tomlinson, B. E. (1977) Lancet 1, 189
12. Bartus, R. T., Dean, R. L., 3rd, Beer, B., and Lippa, A. S. (1982) Science 217, 408–414
13. Bartus, R. T. (2000) Exp. Neurol. 163, 495–529
14. Wang, H. Y., Lee, D. H., D'Andrea, M. R., Peterson, P. A., Shank, R. P., and Reitz, A. B. (2000) J. Biol. Chem. 275, 5626–5632
15. Petit, D. L., Shao, Z., and Yackle, J. L. (2001) J. Neurosci. 21, RC120
16. Liu, Q., Kawii, H., and Berg, D. K. (2001) Proc. Natl. Acad. Sci. U. S. A. 98, 4734–4739
17. Seguela, P., Wadiche, J., Dineley-Miller, K., Dani, A. J., and Patrick, J. W. (1993) J. Neurosci. 13, 596–604
18. Meyer, E. M., Kuryatov, A., Gerzanich, V., Lindstrom, J., and Papke, R. L. (1998) J. Pharmacol. Exp. Ther. 287, 918–925
19. Dineley, K. T., Wadiche, J., Dani, J. A., and Patrick, J. W. (2000) Mol. Pharmacol. 47, 164–171
20. de Fiebre, C. M., Meyer, E. M., Henry, J. C., Muraskin, S. L., Rem, W. R., and Papke, R. L. (1995) Mol. Pharmacol. 47, 164–171
21. Burdick, D., Soreghan, B., Kwon, M., Ash, K. H., and Sweatt, J. D. (2001) J. Neurosci. 21, 4125–4133
22. Yang, F., and Cole, G. (1996) Science 274, 109–102
23. Kim, J. J., Rosen, R. A., and Fasulo, M. S. (1993) Behav. Neurosci. 107, 1093–1098
24. Phillips, R. G., and LeDoux, J. E. (1993) Behav. Neurosci. 107, 274–285
25. Atkins, C. M., Selcher, J. C., Petrata, J. J., Trzaskos, J. M., and Sweatt, J. D. (1998) Nat. Neurosci. 1, 602–609
26. Blum, S., Moore, A. N., Adams, F., and Dash, P. K. (1999) J. Neurosci. 19, 3535–3544
27. Selcher, J. C., Atkins, C. M., Trzaskos, J. M., Paylor, R., and Sweatt, J. D. (1999) Learn. Mem. (Cold Spring Harb.) 6, 478–490
28. Papke, R. L., Bertrand, D., Galzi, J. L., DeVillers-Thiry, A., Mulle, C., Hussey, N., Bertrand, S., Ballivet, M., and Changeux, J. P. (1991) Nature 353, 846–849
29. Bertrand, D., DeVillers-Thiry, A., Revah, F., Galzi, J. L., Hussey, N., Mulle, C., Bertrand, S., Ballivet, M., and Changeux, J. P. (1992) Proc. Natl. Acad. Sci. U. S. A. 89, 1261–1265
30. Wang, H. Y., Lee, D. H., Davis, C. B., and Shank, R. P. (2000) J. Neurochem. 78, 1153–1161
31. Price, D. L., Tanzi, R., Borchelt, D. R., and Sisodia, S. S. (1998) Annu. Rev. Genet. 32, 461–493
32. Dineley, K. T., Xia, X., Bui, D., Sweatt, J. D., and Zheng, H. (2002) J. Biol. Chem. 277, 22768–22770
β-Amyloid Peptide Activates α7 Nicotinic Acetylcholine Receptors Expressed in Xenopus Oocytes

Kelly T. Dineley, Karen A. Bell, Duy Bui and J. David Sweatt

J. Biol. Chem. 2002, 277:25056-25061.
doi: 10.1074/jbc.M200066200 originally published online April 30, 2002

Access the most updated version of this article at doi: 10.1074/jbc.M200066200

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 34 references, 18 of which can be accessed free at http://www.jbc.org/content/277/28/25056.full.html#ref-list-1