

Beta Amyloid-Induced Depression of Hippocampal Long-Term Potentiation Is Mediated through the Amylin Receptor

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Alzheimer's disease (AD) is characterized by accumulation of amyloid- β peptide ($A\beta$) in the brain regions that subserve memory and cognition. The amylin receptor is a potential target receptor for expression of the deleterious actions of soluble oligomeric $A\beta$ species. We investigated whether the amylin receptor antagonist, AC253, neutralizes the depressant effects of $A\beta_{1-42}$ and human amylin on hippocampal long-term potentiation (LTP). Furthermore, we examined whether depressed levels of LTP observed in transgenic mice, which overexpress amyloid precursor protein (TgCRND8), could be restored with AC253. In mouse hippocampal brain slices, field EPSPs were recorded from the stratum radiatum layer of the CA1 area (cornu ammonis 1 region of the hippocampus) in response to electrical stimulation of Schaeffer collateral afferents. LTP was induced by 3-theta burst stimulation protocols. $A\beta_{1-42}$ (50 nM) and human amylin (50 nM), but not $A\beta_{42-1}$ (50 nM), depressed LTP evoked using both stimulation protocols. Preapplication of AC253 (250 nM) blocked $A\beta$ - and human amylin-induced reduction of LTP without affecting baseline transmission or LTP on its own. In contrast to wild-type controls, where robust LTP is observed, 6- to 12-month-old TgCRND8 mice show blunted LTP that is significantly enhanced by application of AC253. Our data demonstrate that the effects of $A\beta_{1-42}$ and human amylin on LTP are expressed via the amylin receptor, and moreover, blockade of this receptor increases LTP in transgenic mice that show increased brain amyloid burden. Amylin receptor antagonists could serve as potentially useful therapeutic agents in AD.

Introduction

Alzheimer's disease (AD) represents one of the most serious health and societal burdens of our time, yet at present, there is no proven treatment beyond symptomatic therapies. The accumulation and deposition of amyloid- β peptide ($A\beta$), a 39–43 aa peptide, in brain areas critical for cognition and memory is an important factor in the pathogenesis of AD (Selkoe, 2008). Large, insoluble deposits of $A\beta$ in plaque form coexist with small, diffusible soluble oligomers of the peptide in cortical and limbic regions of AD brains. Soluble $A\beta$ oligomers, composed of dimers, trimers, tetramers and higher order assemblies, consistently inhibit hippocampal long-term potentiation (LTP), a surrogate *in vitro* cellular model for memory and learning (Walsh et al., 2002). Furthermore, naturally secreted $A\beta$ oligomers potently inhibit hippocampal LTP *in vivo*, which is reversible by passive infusions of

either $A\beta$ antibodies or administration of an active $A\beta$ vaccine (Klyubin et al., 2005). Tg2576 mice, which overexpress mutant amyloid precursor protein (APP), when treated with a γ -secretase inhibitor to reduce $A\beta$ levels, demonstrate a profound restoration of impaired LTP (Townsend et al., 2010). Thus, understanding the mechanisms whereby soluble $A\beta$ oligomers can disrupt synaptic function is of great interest in preventing AD progression. Multiple candidate receptors, located pre- or postsynaptically, have been implicated in mediating $A\beta$ disruption of synaptic processes and are potential targets for developing anti- $A\beta$ therapies (Huang and Mucke, 2012).

Of these receptors, we have identified the amylin receptor as a target for the neuronal actions of $A\beta$ (Jhamandas et al., 2011). Human amylin (h-Amylin (islet amyloid polypeptide)) is a 37-residue amyloidogenic peptide first isolated from pancreatic islets of Langerhans in patients with type 2 diabetes (Cooper et al., 1987). Human amylin acts via a G-protein-coupled receptor (amylin receptor) composed of the calcitonin receptor (CTR) and a receptor-associated membrane protein (RAMP) and demonstrates a profile of neurotoxicity that is strikingly similar to that of $A\beta$ (Jhamandas et al., 2011). Thus, blockade of the amylin receptor with an antagonist (AC253) attenuates electrophysiological and neurotoxic actions of $A\beta$ on human and rat fetal neurons and downregulation of the amylin receptor using siRNA blunts $A\beta$ toxicity. Further, we have shown it is the amylin-3 (CTR + RAMP3 = AMY3), but not AMY1 or AMY2, subtype of the amylin receptor that is most relevant for the activation of

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intracellular cAMP-dependent pathways and expression of A β and h-Amylin toxicity (Fu et al., 2012). Collectively, these observations raise the important question of whether the amylin receptor is also a target for the expression of deleterious effects of A β on synaptic plasticity and learning.

Herein, we sought to examine whether A β -induced reduction of LTP is also observed for h-Amylin, and if so, are such effects expressed via the amylin receptor. Furthermore, we investigated the basis for depressed LTP observed in APP overexpressing TgCRND8 mice with the notion that LTP in such mice could be restored (to levels observed in age-matched wild-type mice) by pharmacologically blocking the amylin receptor.

Materials and Methods

Mouse lines. For initial set of experiments examining the effects of A β and h-Amylin on LTP, we used 2- to 3-month-old male or female C57BL/6 mice. For experiments investigating the role of the amylin receptor in LTP in mice overexpressing APP, we used transgenic (Tg) CRND8 mice (human APP695 transgene array incorporating Swedish K670M/N671L and Indiana V717F mutations superimposed upon a C57BL6/C3H genetic background that have been described previously to exhibit A β plaques and cognitive defects by 3 months of age (Chishti et al., 2001). Specifically we used 6- to 12-month-old TgCRND8 and age-matched, wild-type littermate (C57BL/6 background) male or female mice. All experiments were performed in compliance with the relevant laws and guidelines set by the Canadian Council for Animal Care and with the approval of the Human Research Ethics Board and Animal Care Use Committee (Health Sciences) at the University of Alberta.

Slice preparation and electrophysiology. Brains were quickly removed from mice following decapitation, placed in cold artificial CSF (aCSF) on a vibratome chamber and transverse sections cut through the hippocampus. The aCSF contained (in mM) 124 NaCl, 3 KCl, 2.4 CaCl₂, 2 MgCl₂, 1.25 NaH₂PO₄, 26 NaHCO₃ and 10 D-glucose, and was equilibrated with 95% O₂ and 5% CO₂. Hippocampal slices (400 μ m thick) were maintained in an aCSF-filled holding chamber at room temperature and transferred to the submerged recording chamber constantly perfused with aCSF (2 ml/min) at 30°C. Field EPSP (fEPSP) was recorded with a metallic (Pt/Ir) electrode (FHC, Inc.) from the stratum radiatum layer of cornu ammonis 1 region of the hippocampus (CA1) area, and the Schaffer collateral afferents were stimulated with 100 μ s test pulses via a bipolar cluster electrode (FHC, Inc.) (Kimura and Ohno, 2009). To evaluate basal synaptic transmission, we applied different stimulation strengths (125–300 μ A in steps of 25 μ A) and plotted the amplitudes of presynaptic fiber volleys versus the corresponding fEPSP slopes to compare the slope of input–output (*I/O*) curves of fEPSP. For LTP experiments, the stimulus strength was set to elicit 40–50% of the maximum fEPSP amplitude and test pulses delivered to Schaffer collaterals once every 30 s. LTP was induced by a 3 theta burst stimulation (3-TBS) protocol (each burst consisted of 4 pulses at 100 Hz with a 200 ms interburst interval). Before 3-TBS or drug application, the responses were monitored for at least 10 min to ensure a stable baseline of fEPSP. To determine whether the magnitude of LTP differed significantly between groups, average responses during the last 20 min block of recordings (40–60 min after TBS) were compared. To test paired-pulse facilitation, we measured the percentage increase in slope of the fEPSP relative to the first one with different interpulse intervals (20–500 ms).

Drugs and their application. Soluble oligomeric A β _{1–42}, the reverse nonfunctional sequence peptide A β _{42–1}, and h-Amylin were used in the present study and prepared as per previously published protocols (Stine et al., 2003; Jhamandas et al., 2011). A β _{1–42} and A β _{42–1} were purchased from rPeptide, and h-Amylin and AC253 were purchased from American Peptide. All drugs and chemicals were applied directly to the slice via bath perfusion, which allowed for a complete exchange of the perfusate in less than a minute and a half.

Immunohistochemistry. Following electrophysiological recordings, hippocampal brain slices from TgCRND8 and control wild-type mice were fixed with 4% paraformaldehyde for immunohistochemical analysis of A β plaque deposition (for details, see Jhamandas et al., 2011).

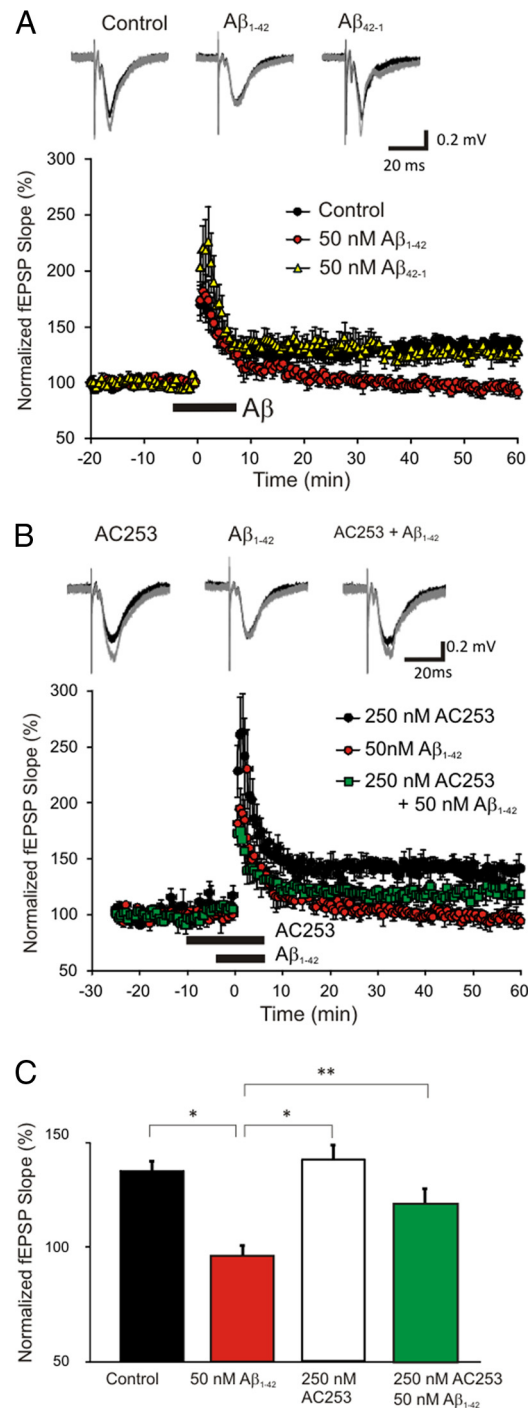


Figure 1. Amylin receptor antagonist AC253 reverses A β _{1–42}-induced depression of hippocampal LTP. **A**, A 50 nM concentration of A β _{1–42}, but not A β _{42–1}, impaired LTP in hippocampal slices. LTP was induced by 3 \times TBS protocols at Schaffer collateral–CA1 synapses. A β _{1–42} or A β _{42–1} was perfused 5 min before and after 3 \times TBS. **B**, AC253 (250 nM) reverses A β _{1–42}-induced depression of hippocampal LTP. AC253 was perfused 5 min before 50 nM A β _{1–42} application and LTP was induced by 3 \times TBS protocols in the presence of AC253 and A β _{42–1}. Each point indicates the fEPSP slope normalized to the average baseline response over 10 min before drug is applied; the bars indicate onset and duration of application. All data are presented as mean \pm SEM. Traces are the average of fEPSPs recorded during baseline (black) and 40–60 min after 3 \times TBS (gray). Calibration: 0.2 mV, 20 ms ($n = 4–6$ mice per group). **C**, The summary bar graphs show significant reductions in LTP as measured by 50 nM A β _{1–42} but not 50 nM A β _{1–42} with 250 nM AC253. * $p < 0.01$ for control vs A β _{1–42} and AC253 vs A β _{42–1}, and ** $p < 0.05$ for A β _{1–42} vs A β _{1–42} with AC253; ANOVA Tukey's *post hoc* test.

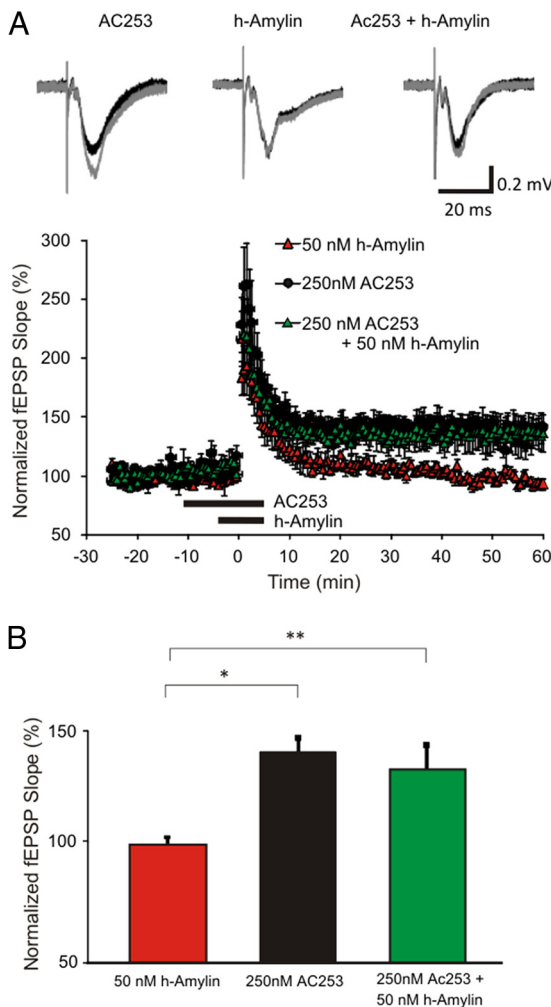


Figure 2. AC253 reverses h-Amylin-induced depression of hippocampal LTP. **A**, AC253 (250 nM) was perfused 5 min before 50 nM h-Amylin application and LTP was induced by 3 \times TBS protocols in the presence of AC253 and h-Amylin. Each point indicates the fEPSP slope normalized to the average baseline response over 10 min before drug is applied; the bars indicate onset and duration of application. All data are presented as mean \pm SEM. Traces are the average of fEPSPs recorded during baseline (black) and 40–60 min after 3 \times TBS (gray). Calibration: 0.2 mV, 20 ms ($n = 4–6$ mice per group). **B**, The summary bar graphs show significant reductions in LTP with application of 50 nM h-Amylin, but not 50 nM h-Amylin with 250 nM AC253. * $p < 0.01$ for AC253 vs h-Amylin, and ** $p < 0.05$ for h-Amylin vs h-Amylin with AC253; ANOVA Tukey's *post hoc* test).

Briefly, 25- μ m-thick hippocampal brain slices were sectioned, and free-floating sections were immunohistochemically stained with 6E10 mouse monoclonal antibody (Signet Laboratories) and visualized with Alexa Fluor 546 anti-mouse antibody (Invitrogen).

Statistics. Results were expressed as means \pm SE. Statistical analysis was performed using one-way ANOVA followed by *post hoc* Tukey's honestly significant difference (HSD) test (for multiple comparisons) or unpaired *t* test (for pairwise comparisons).

Results

Effects of $A\beta_{1-42}$ on hippocampal LTP are expressed via the amylin receptor

Application of soluble oligomeric $A\beta_{1-42}$ (50 nM) depressed LTP induced by a weak tetanization protocol at the CA1 region of wild-type mouse hippocampal slices. In contrast, using the same protocol of drug application (for 5 min before and following 3-TBS), equimolar concentrations of the reverse peptide, $A\beta_{42-1}$, did not cause any significant alteration in LTP (Fig. 1A). LTP was

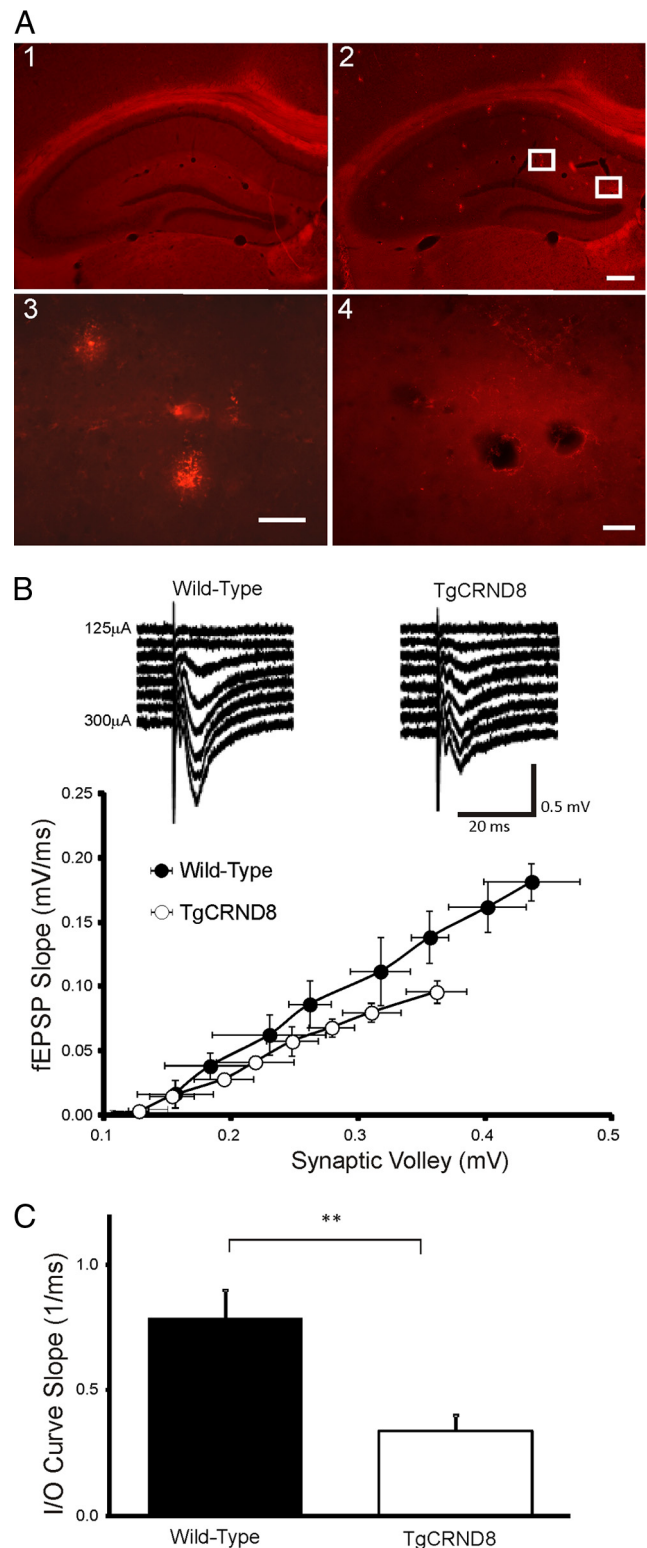


Figure 3. Impairments of basal synaptic transmission in hippocampal slices from TgCRND8 mice. **A**, Immunohistochemical staining for amyloid within the hippocampus of wild-type (1) and TgCRND8 (2) mice. Boxed areas in 2 are shown at higher magnification in 3 and 4. In 3, amyloid plaques are visualized within the brain parenchyma, and in 4, amyloid deposits are observed within walls of blood vessels (seen as black holes). Scale bars: 1, 2 (in 2), 200 μ m; 3, 4, 20 μ m. **B**, I/O curves relating the amplitude of the presynaptic fiber volley to the slope of fEPSP at various stimulus intensities (125–300 μ A in 25 μ A increments). Traces are fEPSP responses at each stimulus intensity. **C**, The summary bar graph shows significant reductions in basal synaptic transmission as measured by the average slope of I/O curves in TgCRND8 mice vs wild-type controls ($n = 7$ mice per each group, ** $p < 0.05$; unpaired *t* test).

impaired with 50 nM A β_{1-42} ($p < 0.01$; unpaired t test) but not A β_{42-1} ($p = 0.3148$; unpaired t test) during the 40–60 min period after induction (Fig. 1*A, C*). Application of A β_{1-42} in the period after LTP induction had no effect (data not shown). To determine whether A β_{1-42} -induced reduction in LTP is expressed amylin receptors, we used AC253 (LGRL SQELHRLQTYPRNTGNTY), a polypeptide that is a potent amylin receptor antagonist. We applied 250 nM AC253 continuously for 5 min before exposure of the hippocampal slices to 50 nM A β_{1-42} and for 5 min after LTP induction. The concentration of AC253 used was selected based on prior studies that examined the ability of this antagonist to block h-Amylin and A β in rat and human fetal neurons and HEK293 cells transfected with subtypes of amylin receptors (Jhamandas and MacTavish, 2004; Jhamandas et al., 2011; Fu et al., 2012). AC253, applied alone did not affect control LTP (Fig. 1*B, C*; $p = 0.9189$, Tukey's HSD test). However, application of AC253 resulted in a significant restoration of hippocampal LTP observed following exposure of the brain slices A β_{1-42} (Fig. 1*B, C*; $p < 0.05$, Tukey's HSD test).

Human amylin induces depression of hippocampal LTP

We further investigated whether application of h-Amylin also causes the basal depression of hippocampal LTP induced by 3-TBS protocol in a manner similar to that induced by A β_{1-42} . Human amylin (50 nM) application also resulted in impaired hippocampal LTP (Fig. 2*A*; $p < 0.01$, unpaired t test), which could be reversed with preapplication of AC253 (250 nM; Fig. 2*A, B*; $p < 0.05$, Tukey's HSD test).

Hippocampal synaptic disruption in TgCRND8 mice

To identify a potential role for the amylin receptor in an animal model where cognitive and memory deficits are attributable to presence of significant brain levels of A β , we examined hippocampal LTP in TgCRND8 mice that overexpress mutant human APP695. TgCRND8 mice at 6 months and older demonstrate marked amyloid deposition in the hippocampus and cortex than age-matched wild-type littermate controls (Fig. 3*A*). In hippocampal slices from 6- to 12-month-old TgCRND8, I/O curves of fEPSPs in response to different stimulation strengths were significantly different from those of wild-type controls (Fig. 3*B*). Consequently, basal synaptic transmission as assessed by the average slope of I/O curves was reduced in TgCRND8 mice ($p = 0.0063 < 0.01$; unpaired t test; Fig. 3*C*). However, we were able to evoke LTP at hippocampal Schaffer collateral-CA1 synapses of both wild-type and hemizygous transgenic mice as demonstrated in Figure 4*B*. LTP induced by 3-TBS was significantly depressed during 40–60 min after its induction in TgCRND8 mice compared with wild-type age-matched controls ($p < 0.05$; unpaired t test). Therefore, basal excitatory synaptic transmission (as assessed by I/O

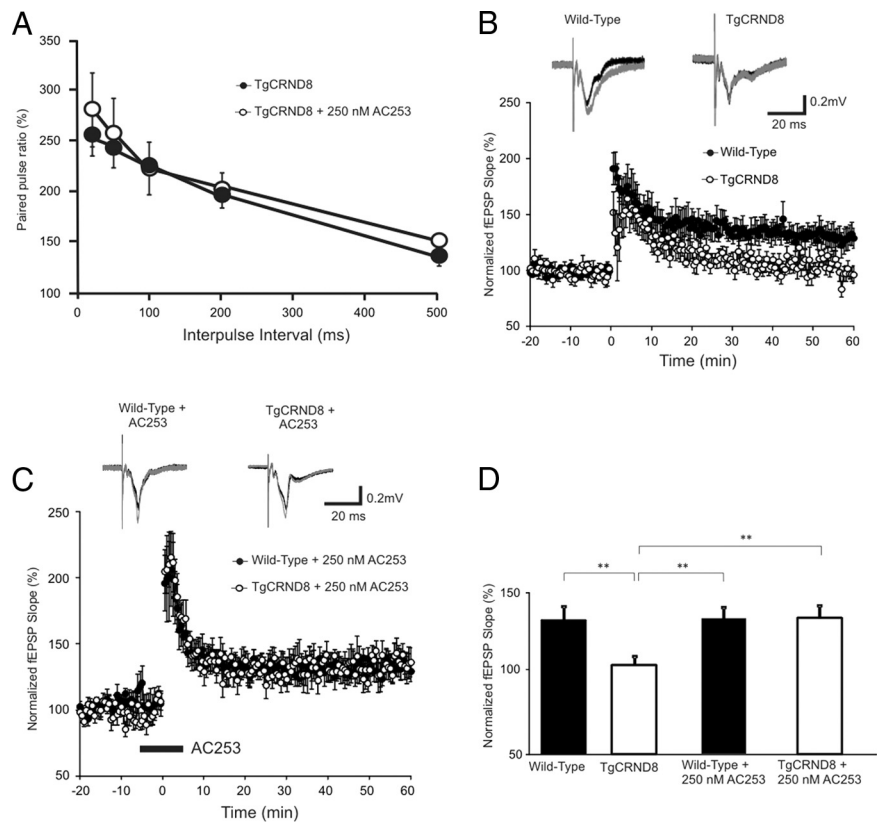


Figure 4. Impairment of hippocampal LTP in TgCRND8 mice is reversed with AC253. **A**, Paired-pulse facilitation across different interstimulus intervals (20–500 ms) under control conditions in TgCRND8 mice and in the presence of 250 nM AC253 ($n = 5$ mice for each interstimulus interval). **B**, LTP was induced by 3 \times TBS protocols in wild-type and TgCRND8 mice. Each point indicates the fEPSP slope normalized to the average baseline response before 3 \times TBS at time 0. **C**, LTP was induced by 3 \times TBS protocols in the presence of 250 nM AC253. AC253 was perfused 5 min before and after 3 \times TBS. Each point indicates the fEPSP slope normalized to the average baseline response over 10 min before AC253 is applied; the bars indicate onset and duration of application. All data are presented as mean \pm SEM. Traces are the average of fEPSPs recorded during baseline (black) and 40–60 min after 3 \times TBS (gray) ($n = 6$ mice for wild-type group and $n = 10$ mice for TgCRND8 group). **D**, The summary bar graphs show significant reductions in LTP as measured by the average normalized fEPSP slopes during 30–40 min after 3 \times TBS in TgCRND8 mice but not with 250 nM AC253. ** $p < 0.05$ for TgCRND8 mice vs wild-type control, wild-type control with AC253 and TgCRND8 mice with AC253 (ANOVA Tukey's *post hoc* test).

relationships) and LTP at hippocampal CA1 are reduced in TgCRND8 mice, as reported in other AD transgenic mouse models (for reviews see Rowan et al., 2003; Wilcox et al., 2011).

A β depression of hippocampal LTP in TgCRND8 mice is amylin receptor dependent

First, we tested the effects of AC253 on paired-pulse facilitation which represents a short-term form of synaptic plasticity reflecting presynaptic transmitter release probability. Paired-pulse facilitation was indistinguishable between TgCRND mice and controls at >6 months of age (Fig. 4*A*). We next examined whether LTP at hippocampal Schaffer collateral-CA1 synapses of TgCRND8 mice is dependent on the amylin receptor. Preapplication of AC253 (250 nM) to hippocampal slices for 5 min before and after 3-TBS, did not affect LTP during 40–60 min after induction in wild-type mice ($p = 0.9999$, Tukey's HSD test, Fig. 4*B*). However, basal depression of LTP in TgCRND8 is restored by application of 250 nM AC253 ($p < 0.05$, Tukey's HSD test) to comparable levels that are observed in age-matched wild-type littermate mice ($p = 0.9997$, Tukey's HSD test) (Fig. 4*C, D*). Furthermore, in TgCRND mice, AC253 applied without TBS had no effect on fEPSPs (data not shown).

Discussion

In this study we report that the amyloidogenic protein h-Amylin, like soluble oligomeric A β , inhibits hippocampal LTP and both the h-Amylin- and A β -induced impairment of LTP is blocked by the amylin receptor antagonist, AC253. Further, in the presence of AC253, depression of LTP in transgenic mice that overexpress APP (Tg CRND8) reported by others (Bellucci et al., 2006; Arrieta-Cruz et al., 2010), and confirmed by us, is reversed to levels seen in wild-type, age-matched control littermate mice. The notion that h-Amylin is capable of disrupting synaptic plasticity seems at first inconsistent with the multiple basic homeostatic regulatory functions ascribed to this endogenous peptide. However, available data indicate that amylin effects may be concentration dependent. The usual plasma concentration for h-Amylin is in the low picomolar range, even following stimuli that promote a release of the peptide in response to glucose load (Butler et al., 1990). At these concentrations, which regulate feeding and energy metabolism, the peptide seems nontoxic, and it is only at higher concentrations (nanomolar to micromolar range) that h-Amylin seems to exert its toxic effects (Fu et al., 2012).

Multiple receptors have been postulated as candidates for the expression of biological effects of A β in *in vitro* and *in vivo* models that recapitulate, in part, features of AD pathology (Patel and Jhamandas, 2012). Of these, we have focused on the amylin receptor, which comprises of a combination of CTR and RAMP, serves as a target receptor for human amylin, and has a widespread CNS distribution including within the rodent and primate hippocampus (Sexton et al., 1994; Paxinos et al., 2004). Moreover, discrete peptide sequences of A β and amylin have been identified as interfaces for self- and cross-interaction between the two peptides (Andreetto et al., 2010). We have identified that the direct effects of A β on human and rat fetal neurons are expressed via the amylin receptor, and furthermore, data from HEK293 cells transfected with subtypes of amylin receptors suggests that it is the AMY3 rather than AMY1 (CTR + RAMP1) or AMY2 (CTR + RAMP2) receptor that is the specific target for A β (Jhamandas and MacTavish, 2004; Jhamandas et al., 2011; Fu et al., 2012). The present data suggest that in an *in vitro* paradigm of synaptic plasticity (LTP), the depressant effects of low nanomolar concentrations of A β are also expressed via the amylin receptor.

TgCRND8 mice encode a double mutant form of APP695 (KM670/671NL+V717F) under the control of the PrP gene promoter and recapitulate many of the essential features of AD pathology in an accelerated fashion (Chishti et al., 2001). Histological deposition of A β in plaque form is evident in 100% of animals by 3 months of age and dystrophic neuritic changes at hippocampal synapses are present at 5 months of age. These neuropathological changes correlate with cognitive and behavioral impediment that are observed as early as 3 months of age. Our study demonstrates that electrophysiological markers of hippocampal synaptic dysfunction, as assessed by reductions in both basal synaptic transmission (*I/O* curves) and LTP, are present in all TgCRND8 mice by 6 months of age compared with age-matched, wild-type littermates in whom no synaptic impairment was identified. Short-lived presynaptic plasticity as measured by paired pulse facilitation was normal in TgCRND8 and wild-type mice as reported for other mouse models (Chapman et al., 1999; Kimura and Ohno, 2009). Treatment of hippocampal brain slices with AC253 resulted in restoration of LTP recorded from TgCRND8 mice to levels that are observed in wild-type controls. However, in TgCRND8 mice, AC253 had no effect on fEPSPs recorded during application of the drug either before LTP induc-

tion or in the absence of TBS. We and others have shown that total hippocampal A β levels (in soluble oligomeric and plaque form) in TgCRND8 mice >6 months of age are markedly elevated compared with wild-type littermates (Chishti et al., 2001; Jhamandas et al., 2011). We suggest that the increase in ambient A β levels in TgCRND8 mice is responsible for depression of LTP that is observed in these animals as they get older and that blockade of the amylin receptor, which we have shown to serve as a putative target receptor for A β , causes an improvement in LTP. Interestingly, we have also observed that the age-dependent and region-specific (cortex, hippocampus and septum) increases in the amylin receptor (CTR and RAMP3) and A β in TgCRND8 mice are also apparent in cortex and hippocampus in postmortem brain tissue from AD patients compared with age-matched controls (our unpublished observations).

The nature and mechanisms of the soluble oligomeric A β interaction with the amylin receptor, specifically the AMY3 moiety comprising CTR and RAMP3 (Fu et al., 2012) deserve comment. We have previously shown that the locus of such a ligand-receptor interplay is at the level of the postsynaptic membrane where A β , through the amylin receptor, modulates activity of a suite of ionic conductances, mostly potassium channels, that results in increased neuronal excitability, elevations in intracellular calcium levels and activation of cAMP along with other downstream signal transduction mediators (Jhamandas and MacTavish, 2004; Jhamandas et al., 2011; Fu et al., 2012). A β is also capable of synaptic effects by depressing excitatory synaptic transmission in rat brain slices, possibly via metabotropic glutamate receptors (Chin et al., 2007). These events play out on a relatively short time scale (milliseconds to minutes) but have the potential to disrupt intracellular homeostasis and synaptic plasticity. Longer exposures to soluble oligomeric A β has been shown in many *in vitro* paradigms to result in cell death via apoptotic and non-apoptotic mechanisms (Selkoe, 2008; Jhamandas et al., 2011). Thus, application of amylin receptor antagonists or siRNA downregulation of the amylin receptor blunts both the early and late events attributable to the soluble oligomeric A β species. The present study provides first preclinical evidence for restoration of A β -induced impairment of synaptic plasticity using blockade of the amylin receptor and suggest that antagonists for this receptor, originally developed for the treatment of diabetes mellitus, may offer promising therapeutic option for AD.

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