Published in final edited form as:
Neurosci Lett. 2006 July 31; 403(0): 162–165. doi:10.1016/j.neulet.2006.04.065.

Amyloid-beta$_{1–42}$ reduces neuronal excitability in mouse dentate gyrus

Sung Hwan Yun[a,b,c], **Georgi Gamkrelidze**[d], **W. Blaine Stine**[c], **Patrick M. Sullivan**[e], **Joseph F. Pasternak**[a,b,c], **Mary Jo LaDu**[f], and **Barbara L. Trommer**[a,b,c,*]

[a]Departments of Pediatrics and Neurology, Feinberg School of Medicine, Northwestern University, Chicago, IL 60614, United States

[b]Department of Pediatrics, Division of Neurology, Evanston Northwestern Healthcare, 2650 Ridge Avenue, Evanston IL 60201, United States

[c]ENH Research Institute, 1001 University Place Evanston, IL 60202, United States

[d]Department of Physiology and Neuroscience, New York University School of Medicine, New York, NY 10016, United States

[e]Department of Medicine, Division of Neurology, Duke University, Durham, NC 27710, United States

[f]Department of Anatomy and Cell Biology, University of Illinois at Chicago, Chicago, IL 60612, United States

Abstract

Amyloid-β (Aβ) is causally implicated in Alzheimer's disease and neuroplasticity failure has acquired validity as a possible mechanism of early AD pathogenesis. We have previously demonstrated that oligomeric Aβ $_{1–42}$ inhibits LTP in the dentate gyrus of rat hippocampal slices. We now show, using whole cell recordings in hippocampal granule cells, that oligomeric Aβ$_{1–42}$ decreases neuronal excitability. In particular, Aβ$_{1–42}$ application was associated with a decrease in the number of action potentials fired in response to current injection, and with an increase in the amplitude of the afterhyperpolarization. Reduced excitability may underlie the Aβ-mediated impairment in neuroplasticity, and ultimately may contribute to the memory loss in Alzheimer disease.

Keywords

Whole cell recording; Hippocampal slices; Afterhyperpolarization; Alzheimer disease

Amyloid-β may be considered a causal factor in AD because autosomal dominant mutations associated with Aβ overproduction lead to the disease. We and others have specifically demonstrated that oligomeric Aβ$_{1–42}$ induces inhibition of long-term potentiation (LTP) in the rodent hippocampus [13,15–17]. LTP is a form of neuroplasticity that is a widely accepted model for the cellular basis of learning and memory. Lack of neuronal reserve and insufficient neuroplasticity are attractive theories to explain the pathogenesis of AD, with gradual decline in synaptic function eventually leading to frank neuronal loss [6,11].

*Corresponding author. Tel.: +1 847 570 2577; fax: +1 847 570 2073. btrommer@northwestern.edu. .

Based on recent reports demonstrating a crucial role for intrinsic neuronal excitability in the generation of LTP [2], we hypothesized that oligomeric $A\beta_{1–42}$ acts to diminish neuronal excitability. To test this hypothesis we used whole cell recordings in hippocampal dentate gyrus granule cells and compared action potential (AP) generation and afterhyperpolarization (AHP) size in the absence and presence of oligomeric $A\beta_{1–42}$.

Hippocampal slices used in the experiments were prepared from young male (5–6 week) C57Bl/6 mice. Animals were decapitated after deep inhalation anaesthesia by isoflorane, and their brains were quickly removed and placed in ice-cold artificial cerebrospinal fluid (ACSF) of the following composition (in mM): NaCl 124, KCl 3, $CaCl_2$ 2.4, $MgSO_4$ 1.3, NaH_2PO_4 1.25, $NaHCO_3$ 26, and glucose 10 (gassed with 95% O_2/5% CO_2, pH 7.4). After removal of the lateral cerebral convexities by parasagittal cuts, brains were bisected along the interhemispheric fissure, trimmed, and glued at the lateral surface to the specimen tray of a vibrating tissue slicer (Vibratome) with cyanoacrylate. Slices were cut at a thickness of 350 μm and were transferred to a holding chamber where they were kept at 35 °C for 60 min then allowed to cool to room temperature. Slices were transferred as needed to a small volume perfusion chamber maintained at 32 °C, superfused with ACSF at 2 ml/min, and mounted on a fixed stage Zeiss Axioscope with attached infrared camera and video monitor.

Recording electrodes had tip impedence 4.5–6.0 MΩ and the electrode solution consisted of (in mM): potassium gluconate 117.5, potassium methylsulfate, 17.5, NaCl 8, Mg_2 ATP 3, GTP 0.4, EGTA 0.2, and *N*-2-hydroxyethylpiperazine-*N*-2-ethanesulfonic acid (HEPES) 10 (pH 7.2, osmolarity adjusted to 285 mOsm). Liquid junction potentials were determined to be 8 mV for this internal solution and all reported membrane potentials were appropriately corrected. Visualized whole-cell recordings were made in the suprapyramidal limb of the dentate gyrus granule cell (GC) layer approximately midway between the crest and the distal end of the limb using an Axoclamp-2A amplifier and pClamp9 software (Axon Instruments). Cell access was obtained in voltage clamp mode and seals were 2–5 GΩ prior to break-in. Membrane input resistance (Rm) was measured in response to hyperpolarizing pulse of 0.1 nA and was more hyperpolarized than −72 mV for all cells used.

Stable oligomeric $A\beta_{1–42}$ (Aβ) was prepared as previously described [12]. The vehicle for Aβ was DMSO diluted in F12 culture media. Aβ or the identical volume of vehicle was resuspended in ACSF to give a concentration of 300 nM. Final DMSO concentrations in ACSF for recording were always ≤0.01%. Cells were subjected to one of two protocols: perfusion with ACSF followed by oligomeric $A\beta_{1–42}$ or perfusion with ACSF followed by vehicle.

To investigate the excitability of neurons, action potentials (APs) were induced in GCs from $V_{holding}$ = −80 mV in two ways: (1) 100 ms duration current steps ranging from 50 to 250 pA in 50 pA increments; or (2) a single 150 pA current injection of 1 s duration. To test the effect of oligomeric Aβ on excitability, the number of APs generated by these current injections and the amplitude of the corresponding afterhyperpolarizations (AHP) was analyzed before and after 5–6 min bath application of 300 nM oligomeric-$A\beta_{1–42}$ or vehicle. AHP amplitude was taken as the absolute value of the difference between the voltage at threshold and the voltage measured 10 ms after the peak of the action potential using the 1 s duration current injections. For uniformity of comparisons (i.e., to account for the decrease in AP number by oligomeric Aβ, or slight increases or decreases seen in vehicle), AHP measurements were made on the last AP in the condition with the smallest AP number for each cell, and compared with the corresponding AP in the paired condition for that cell. All values are given as mean ± S.E.M. Student's *t*-test was used for statistical comparisons. Unpaired *t*-tests were used to compare groups of cells, and paired *t*-tests were used when sequential measurements were obtained on individual cells in different conditions.

Mean baseline Rm did not differ between Aβ treated (160.1±11.3 MΩ, N=7) and vehicle treated (156.0±10.4 MΩ, N=7) cells at baseline (p>0.7). Cells in both groups showed a small increase in Rm following treatment but did not differ on this measure (172.3±14.3 MΩ versus 182.7±12.1 MΩ for Aβ versus vehicle, p>0.6).

In the 100 ms protocol (Fig. 1), Aβ was associated with a 17.0±6.1% reduction in number of AP elicited by a 250 pA current injection (6.7±0.4 AP in control versus 5.6±0.5 AP in Aβ) and this reduction was significant (N=7, p<0.05). Similarly, using a 150 pA current injection in the 1 s protocol (Fig. 2), Aβ was associated with a 34.3±6.4% reduction in AP number and this change was also significant (15.7±2.5 AP in control versus 10.4±2.1 in Aβ, N=7, p<0.05). In contrast to Aβ, vehicle perfusion had no significant effect on AP number generated by either the 100 ms (7.1±0.3 in control versus 6.7±0.3 in vehicle, N=7, p>0.07) or the 1 s current injection (18.9±2.3 in control versus 17.4±2.0 in vehicle, N=7, p>0.2).

The longer (1 s, 150 pA) current injection was also used to examine the AHP. Representative traces, including enlarged views of AHPs for corresponding AP, are shown in Fig. 2A. As shown in Fig. 2C, the mean AHP amplitude was significantly increased by Aβ (120.4±4.8% of control levels, N=9, p<0.01). In contrast, perfusion of vehicle had no effect on the amplitude of the AHP compared with control ACSF (101.4±4.4% of control levels, N=7, p>0.7).

Together these data confirm that Aβ decreases neuronal excitability as measured by AP number, and demonstrate that this decrease is accompanied by a corresponding increase in AHP amplitude.

The present results show that oligomeric $A\beta_{1–42}$ decreased the number of AP generated by current injection and increased the amplitude of AHP, thus significantly dampening neuronal excitability. These changes in excitability parallel previous indications that Aβ is associated with impairment in neuronal function when LTP is used as the outcome measure [13,16,17].

Recent reports have suggested an association between neuronal excitability and LTP induction. For example, Frick et al. [2] reported that the induction of LTP in rat hippocampal CA1 pyramidal neurons was accompanied by a local NMDA receptor-dependent increase in dendritic excitability as evidenced by increased calcium spike amplitude. Aged mice with enhanced excitability (decreased AHP) on the basis of knockout of the auxiliary potassium channel subunit Kvβ1.1 showed greater LTP magnitude than controls [7]. In rat hippocampal CA1, “priming” via metabotropic glutamate receptor activation led both to increases in excitability (decreased AHP and increased AP number) and facilitation of LTP induction [1]. Our data would suggest that a reduction in neuronal excitability is a plausible explanation for previously reported Aβ-mediated impairments in LTP [13,15–17].

Several prior studies have examined the effects of Aβ on neuronal excitability with a focus on potassium currents. For example, Good et al. [3,4] have demonstrated that Aβ selectively blocks the fast inactivating potassium current (I_A) as well as the delayed rectifier in neuronal culture. Using modeling techniques, these investigators have postulated that blockade of I_A could lead to increased excitability by increasing intracellular calcium. In contrast, others have shown that Aβ increases I_A [8,9]. Upregulation of transient voltage-gated potassium currents can diminish both the probability of AP generation and the magnitude of LTP [5]. Since we find a decrease in AP generation with Aβ, our results would be consistent with an increase rather than a blockade of potassium current.

Another candidate for the mediation of excitability and its modulation by Aβ is the nicotinic acetylcholine receptor (nAChR). Activation of nAChRs by nicotine both enhances neuronal excitability and facilitates LTP [10]. nAChRs have been implicated as mediators of the oligomeric Aβ effect on LTP [14] and are known to be inhibited by Aβ.

In conclusion, our data demonstrate that oligomeric $A\beta_{1-42}$ is associated with dampening effects on neuronal excitability. These effects are likely to contribute to the neuroplasticity impairments, and ultimately to the cognitive impairments, that are associated with AD.

Acknowledgments

Supported by RO1 AG19121 (M J L); RO3-AG022605 (NIA), IIRG-04-1379 (Alzheimer's Association), and a generous donation from the C. Robert Passantino Charitable Remainder Trust (BLT).

References

[1]. Cohen AS, Coussens CM, Raymond CR, Abraham WC. Long-lasting increase in cellular excitability associated with the priming of LTP induction in rat hippocampus. J. Neurophysiol. 1999; 82:3139–3148. [PubMed: 10601447]

[2]. Frick A, Magee J, Johnston D. LTP is accompanied by an enhanced local excitability of pyramidal neuron dendrites. Nat. Neurosci. 2004; 7:126–135. [PubMed: 14730307]

[3]. Good TA, Murphy RM. Effect of beta-amyloid block of the fast-inactivating K+ channel on intracellular Ca2+ and excitability in a modeled neuron. Proc. Natl. Acad. Sci. U.S.A. 1996; 93:15130–15135. [PubMed: 8986775]

[4]. Good TA, Smith DO, Murphy RM. Beta-amyloid peptide blocks the fast-inactivating K+ current in rat hippocampal neurons. Biophys. J. 1996; 70:296–304. [PubMed: 8770205]

[5]. Johnston D, Christie BR, Frick A, Gray R, Hoffman DA, Schexnayder LK, Watanabe S, Yuan LL. Active dendrites, potassium channels and synaptic plasticity. Philos. Trans. R. Soc. Lond. B Biol. Sci. 2003; 358:667–674. [PubMed: 12740112]

[6]. Mesulam M-M. Neuroplasticity failure in Alzheimer's disease: bridging the gap between plaques and tangles. Neuron. 1999; 24:521–529. [PubMed: 10595506]

[7]. Murphy GG, Fedorov NB, Giese KP, Ohno M, Friedman E, Chen R, Silva AJ. Increased neuronal excitability, synaptic plasticity, and learning in aged Kvbeta1.1 knockout mice. Curr. Biol. 2004; 14:1907–1915. [PubMed: 15530391]

[8]. Plant LD, Webster NJ, Boyle JP, Ramsden M, Freir DB, Peers C, Pearson HA. Amyloid beta peptide as a physiological modulator of neuronal 'A'-type K(+) current. Neurobiol. Aging. Nov 3.2005 [Epub ahead of print].

[9]. Ramsden M, Plant LD, Webster NJ, Vaughan PF, Henderson Z, Pearson HA. Differential effects of unaggregated and aggregated amyloid beta protein (1–40) on K(+) channel currents in primary cultures of rat cerebellar granule and cortical neurones. J. Neurochem. 2001; 79:699–712. [PubMed: 11701773]

[10]. Salgado-Commissariat D, Rosenfield DB, Helekar SA. Nicotine-mediated plasticity in robust nucleus of the archistriatum of the adult zebra finch. Brain Res. 2004; 1018:97–105. [PubMed: 15262210]

[11]. Selkoe DJ. Alzheimer's disease is a synaptic failure. Science. 2002; 298:789–791. [PubMed: 12399581]

[12]. Stine WB Jr. Dahlgren KN, Krafft GA, LaDu MJ. In vitro characterization of conditions for amyloid-beta peptide oligomerization and fibrillogenesis. J. Biol. Chem. 2003; 278:11612–11622. [PubMed: 12499373]

[13]. Trommer BL, Shah C, Yun SH, Gamkrelidze G, Pasternak ES, Blaine SW, Manelli A, Sullivan P, Pasternak JF, LaDu MJ. ApoE isoform-specific effects on LTP: blockade by oligomeric amyloidbeta1–42. Neurobiol. Dis. 2005; 18:75–82. [PubMed: 15649697]

[14]. Turner PR, O'Connor K, Tate WP, Abraham WC. Roles of amyloid precursor protein and its fragments in regulating neural activity, plasticity and memory. Prog. Neurobiol. 2003; 70:1–32. [PubMed: 12927332]

[15]. Walsh DM, Klyubin I, Fadeeva JV, Cullen WK, Anwyl R, Wolfe MS, Rowan MJ, Selkoe DJ. Naturally secreted oligomers of amyloid beta protein potently inhibit hippocampal long-term potentiation in vivo. Nature. 2002; 416:535–539. [PubMed: 11932745]

[16]. Wang HW, Pasternak JF, Kuo H, Ristic H, Lambert MP, Chromy B, Viola KL, Klein WL, Stine WB, Krafft GA, Trommer BL. Soluble oligomers of beta amyloid (1–42) inhibit long-term potentiation but not long-term depression in rat dentate gyrus. Brain Res. 2002; 924:133–140. [PubMed: 11750898]

[17]. Wang Q, Walsh DM, Rowan MJ, Selkoe DJ, Anwyl R. Block of long-term potentiation by naturally secreted and synthetic amyloid beta-peptide in hippocampal slices is mediated via activation of the kinases c-Jun *N*-terminal kinase, cyclin-dependent kinase 5, and p38 mitogen-activated protein kinase as well as metabotropic glutamate receptor type 5. J. Neurosci. 2004; 24:3370–3378. [PubMed: 15056716]

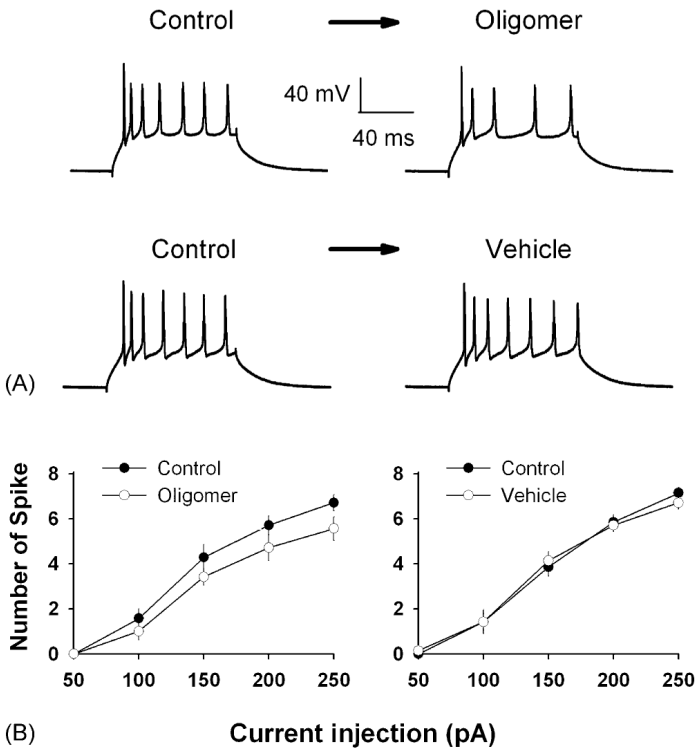

Fig. 1.

Oligomeric-$A\beta_{1-42}$ decreases neuronal excitability: (A) representative records of action potentials evoked by current injection (250 pA, 100 ms). The number of AP was markedly reduced by application of 300 nM oligomer (upper panel) but not by application of vehicle (lower panel); and (B) AP number increases with increasing current intensity under all conditions. Bath application of oligomeric Aβ decreases the AP number at each current intensity compared with control ACSF, whereas application of vehicle does not.

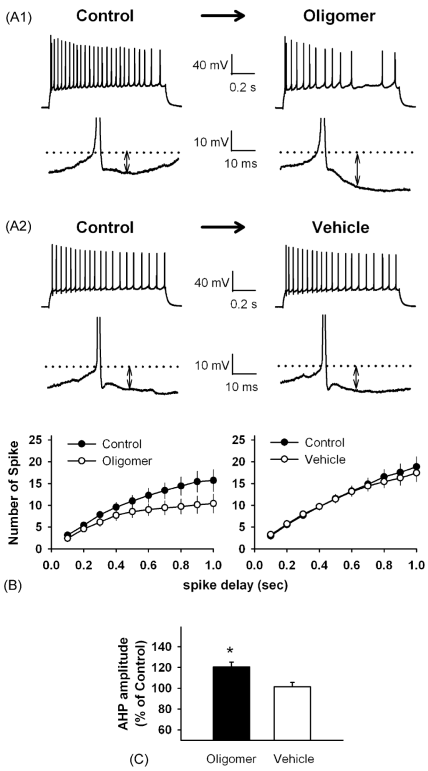

Fig. 2.

Oligomeric-Aβ_{1-42} decreases AP number and increases AHP amplitude: (A) representative records of AP evoked by current injection (150 pA, 1 s). Bath application of Aβ (Panel A1, top) but not vehicle (Panel A2, top) is associated with a decrease in AP number compared with control ACSF. Insets below these tracings show, respectively, expanded detail for spike number 13 in both control and Aβ (A1) and for spike number 19 in both control and vehicle conditions (A2) to demonstrate that Aβ increases AHP amplitude whereas vehicle does not; (B) summary plots of AP number versus delay from onset of current injection. Wash in of Aβ decreases the number of AP (left panels) whereas vehicle wash in has no effect on AP number (right panels); and (C) summary graphs of AHP changes as a function of control AHP amplitude. Wash in of Aβ (filled bars) significantly increases the mean AHP amplitude, whereas vehicle wash in (open bars) has no effect. *p<0.01 (oligomer vs. control).