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Amylin receptor ligands reduce the pathological cascade of Alzheimer's disease

Haihao Zhu, M.D., Ph.D. [a, **], Xiehua Xue, M.D. [a, h], Erming Wang, Ph.D. [a], Max Wallack, B.A. [a, g], Hana Na, Ph.D. [a], Jacob M. Hooker, Ph.D. [f, g], Neil Kowall, M.D., Ph.D. [b, d], Qiushan Tao, Ph.D. [a], Thor D. Stein, M.D., Ph.D. [c, d], Benjamin Wolozin, M.D., Ph.D. [a, b], Wei Qiao Qiu, M.D., Ph.D. [a, d, e, *]

[a] Department of Pharmacology and Experimental Therapeutics, Boston University School of Medicine, Boston, MA, USA
[b] Department of Neurology, Boston University School of Medicine, Boston, MA, USA
[c] Department of Pathology, Boston University School of Medicine, Boston, MA, USA
[d] Alzheimer's Disease Center, Boston University School of Medicine, Boston, MA, USA
[e] Department of Psychiatry, Boston University School of Medicine, Boston, MA, USA
[f] Martinos Center for Biomedical Imaging, Massachusetts General Hospital, Boston, USA
[g] Harvard Medical School, USA
[h] Affiliated Rehabilitation Hospital of Fujian, TCM University, China

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ABSTRACT

Amylin is an important gut-brain axis hormone. Since amylin and amyloid-β peptide (Aβ) share similar β sheet secondary structure despite not having the same primary sequences, we hypothesized that the accumulation of Aβ in the brains of subjects with Alzheimer's disease (AD) might compete with amylin for binding to the amylin receptor (AmR). If true, adding exogenous amylin type peptides would compete with Aβ and reduce the AD pathological cascade, improving cognition. Here we report that a 10-week course of peripheral treatment with human amylin significantly reduced multiple different markers associated with AD pathology, including reducing levels of phospho-tau, insoluble tau, two inflammatory markers (Iba1 and CD68), as well as cerebral Aβ. Amylin treatment also led to improvements in learning and memory in two AD mouse models. Mechanistic studies showed that an amylin receptor antagonist successfully antagonized some protective effects of amylin *in vivo*, suggesting that the protective effects of amylin require interaction with its cognate receptor. Comparison of signaling cascades emanating from AmR suggest that amylin electively suppresses activation of the CDK5 pathway by Aβ. Treatment with amylin significantly reduced CDK5 signaling in a receptor dependent manner, dramatically decreasing the levels of p25, the active form of CDK5 with a corresponding reduction in tau phosphorylation. This is the first report documenting the ability of amylin treatment to reduce tauopathy and inflammation in animal models of AD. The data suggest that the clinical analog of amylin, pramlintide, might exhibit utility as a therapeutic agent for AD and other neurodegenerative diseases.

1. Introduction

Amylin (also known as islet amyloid polypeptide or IAPP) is a highly conserved 37—amino acid peptide that functions as a gut-brain axis hormone (Lutz, 2013; Mietlicki-Baase, 2016; Nishi et al., 1989). Amylin is produced and secreted by the β-cells in pancreas and easily crosses the blood brain barrier (BBB) to bind to its receptor and function in the brain (Banks and Kastin, 1998; Banks et al., 1995; Olsson et al., 2007). The amylin receptor (AmR) is a G-protein coupled receptor (GPCR) (Hay et al., 2015) that mediates several important functions, including regulating glucose metabolism, modulating inflammatory reactions and probably enhancing neurogenesis (Edvinsson et al., 2001; Roth et al., 2013;

* Corresponding author. Department of Psychiatry, Boston University School of Medicine, Boston, MA, USA.
** Corresponding author. Department of Pharmacology & Experimental Therapeutics, Boston University School of Medicine, 72 East Concord Street, R-623, Boston, MA 02118, USA.
E-mail addresses: hhzhu@bu.edu (H. Zhu), wqiu67@bu.edu (W.Q. Qiu).

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- Aβ1-42: DAEFRHDSGYEVHHQKLVFFAEDVGSNKGAIIGLMVGGVVIA
- Amylin: KCNTATCATQRLANFLVH-SSNNFGAIL—SSTNVGSNTY
- AC253 LGRLSQELHRLQTY—PRTNTGSNTY

Fig. 1. Primary amino acid sequences of Aβ, amylin and AC253. Amino acid sequences of Aβ1-42, amylin and AC253 are presented. Identical amino acids between Aβ and amylin are in red; identical amino acid sequences between amylin and AC253 are in blue. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

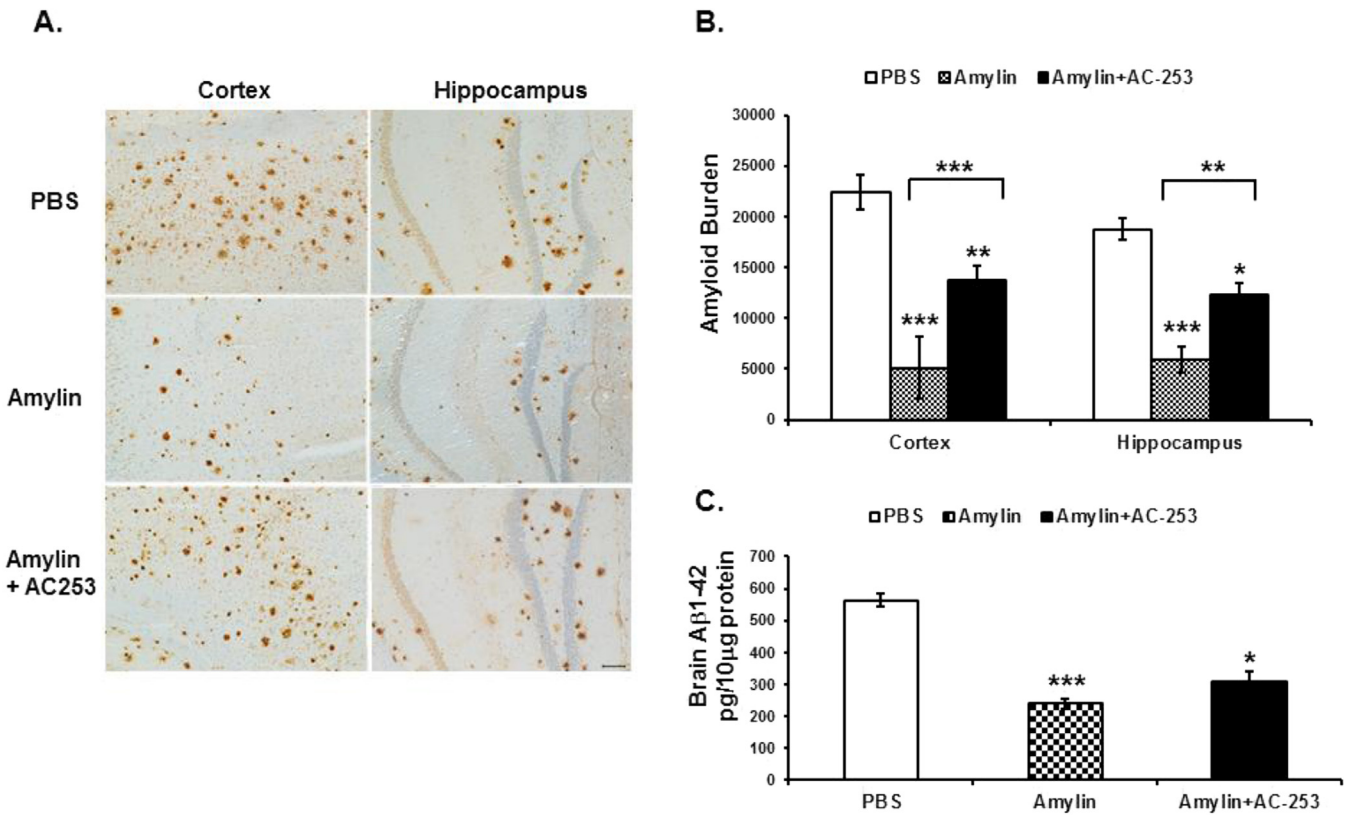

Fig. 2. Amylin treatment reduces amyloid pathology in the brain, and an amylin receptor antagonist attenuates the effect. 5XFAD mice were used to study the effect of amylin treatment on amyloid components of AD pathology in the brain. Each treatment group had 10 mice and total brain proteins were extracted by using TBS-X extraction after completing the treatments. (A) Compared with PBS treatment, the amylin-treated mice had fewer amyloid plaques in the cortex and the hippocampus region as shown by immunostaining with an Aβ antibody, and co-treatment of amylin with AC-253 diminished the effect on the amyloid burden. (B) The amyloid burden for these mice was quantitated and calculated by the number of amyloid plaques x the size of each plaque in the brain regions and showed the group differences by using ANOVA ($p < 0.0001$). (C) Aβ1-42 in the brain extracts was measured by using a specific ELISA and showed the group differences by using ANOVA ($p < 0.0001$). The mean ± SE is shown, and statistical analyses were done on different conditions (ANOVA followed by Tukey test) to compare the PBS treatment and either the amylin or amylin plus AC253 treatment. The groups of amylin and amylin plus AC253 were also compared. Statistical significances $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.0001$.

Trevaskis et al., 2010; Westfall and Curfman-Falvey, 1995).

While an amylin gene polymorphism is associated with cognitive decline in Alzheimer's disease (AD) (Roostaei et al., 2016), increased plasma amylin levels are associated with better cognition in humans (Qiu et al., 2014). Recent studies from two independent laboratories, including our own, find that peripheral treatment with human amylin or its clinically approved analog, pramlintide (Roth et al., 2012), reduced amyloid burden and improved cognitive impairment in multiple mouse models of AD (Adler et al., 2014; Zhu et al., 2015). These positive results raise the possibility that the AmR could be effective targets for therapy of AD.

The mechanism for the beneficial effect of amylin in the AD brain is unclear. Amylin and amyloid-β peptide (Aβ), a major component of AD pathology (Hardy and Selkoe, 2002), share several features, including similar β-sheet secondary structures (Lim et al., 2008), binding to the same AmR (Fu et al., 2012) and being degraded by (Bennett et al., 2003; Qiu et al., 1998; Shen et al., 2006) or bound to insulin degrading enzyme (IDE) (de Tullio et al., 2013). We hypothesized that the elevated levels of Aβ that accumulate in AD could block the AmR, while administering exogenous amylin could provide neuroprotection by competing with Aβ at the AmR (Qiu and Zhu, 2014).

The pathological cascade in AD is thought to proceed from the accumulation of senile amyloid plaques to formation of neurofibrillary tangles with concurrent neuroinflammation, synaptic loss, neuronal death and clinical dementia. If amylin is an effective treatment for AD, the direct effects of amylin should interfere with the AD pathological cascade, inhibiting tauopathy and preventing cognitive decline in humans (Lee et al., 2011). To test whether AmR agonists could delay disease progression, we administered amylin without and with co-injection of its antagonist, AC253 (Coppock et al., 1999), to two different animal models of AD, 5XFAD, which