Interaction between Angiotensin Receptor and β-Adrenergic Receptor Regulates the Production of Amyloid β-Protein

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Alzheimer’s disease (AD) is characterized by the formation of extracellular amyloid plaques containing the amyloid β-protein (Aβ) within the parenchyma of the brain. Aβ is considered to be the key pathogenic factor of AD. Recently, we showed that Angiotensin II type 1 receptor (AT1R), which regulates blood pressure, is involved in Aβ production, and that telmisartan (Telm), which is an angiotensin II receptor blocker (ARB), increased Aβ production via AT1R. However, the precise mechanism underlying how AT1R is involved in Aβ production is unknown. Interestingly, AT1R, a G protein-coupled receptor, was strongly suggested to be involved in signal transduction by heterodimerization with β2-adrenergic receptor (β2-AR), which is also shown to be involved in Aβ generation. Therefore, in this study, we aimed to clarify whether the interaction between AT1R and β2-AR is involved in the regulation of Aβ production. To address this, we analyzed whether the increase in Aβ production by Telm treatment is affected by β-AR antagonist using fibroblasts over-expressing amyloid precursor protein (APP). We found that the increase in Aβ production by Telm treatment was decreased by the treatment of β-AR selective antagonist ICI-118551 more strongly than the treatment of β1-AR selective antagonists. Furthermore, deficiency of AT1R abolished the effect of β2-AR antagonist on the stimulation of Aβ production caused by Telm. Taken together, the interaction between AT1R and β2-AR is likely to be involved in Aβ production.

Key words Alzheimer’s disease; amyloid β-protein; angiotensin receptor; adrenergic receptor

INTRODUCTION

Alzheimer’s disease (AD) is a progressive, fatal neurodegenerative disease characterized clinically by progressive loss of memory and cognitive decline. The neuropathological hallmarks of AD are the accumulation of amyloid β-protein (Aβ) in the extracellular plaques, neurofibrillary tangles made from intracellular abnormally phosphorylated tau, and neuronal loss. Aβ is generated from amyloid precursor protein (APP) through sequential cleavages by two proteases called γ-secretase and β-secretase. Although secreted Aβ40 is much more abundant and 42 residues in length, depending on the site of γ-secretase cleavage. Although secreted Aβ40 is much more abundant than Aβ42, Aβ42 is considered as the causative molecule for triggering the onset of AD because it is more prone to aggregation and is the major component in senile plaques.

Previously, we showed that Angiotensin II type 1 receptor (AT1R), which regulates blood pressure, is involved in Aβ production, and that telmisartan (Telm), an angiotensin II receptor blocker (ARB), increased Aβ production via AT1R. We found that AT1R-knockout mice exhibited a decrease in Aβ accumulation in the brain, due to a decrease in γ-secretase activity. Deficiency of AT1R was found to incompletely generate presenilin complex, which is responsible for γ-secretase activity. Therefore, AT1R is likely to regulate Aβ generation through changing γ-secretase activity. However, the precise mechanism underlying how AT1R is involved in Aβ production is not clear. On the other hand, β2-adrenergic receptor (β2-AR) has also been shown to modulate γ-secretase activity. Very interestingly, AT1R, one of G protein-coupled receptors (GPCRs), was strongly suggested to be regulated in signal transduction by heterodimerization with other GPCR family members, including β2-AR.

GPCRs comprise the largest family of membrane proteins and are the most common target for therapeutic drugs. Surprisingly, over 90% of GPCRs are expressed in the brain, where they appear to have important roles including cognition, mood, synaptic transmission and so on. In addition, GPCRs, classically considered to function as monomers, are actually organized as homodimers and heterodimerize with other GPCR family members. A growing number of observations demonstrate that GPCR oligomerization may occur in native tissues and may have important consequences in receptor function. However, the precise mechanism underlying the regulation of the signal transduction by heterodimers or homodimers of GPCR family members remains to be elucidated. It is also noted that the binding of an agonist or an antagonist to a GPCR possibly promotes a conformational change that results in the activation of receptor-associated heterodimeric G protein and consequent downstream signaling.

Regarding Alzheimer’s disease, GPCRs are reported to be associated with multiple stages of APP proteolysis, including modulation of processing of APP by the α-, β-, and γ-secretase; however, at present, it is not known how these
GPCRs modulate APP processing. Therefore, in this study, in order to know the potential drug targets for the treatment of AD, we aimed to clarify whether the interaction between AT,R and β2-AR is required for the regulation of Aβ production. To address this, we analyzed whether the increase in Aβ production induced by Telm treatment is affected by β2-AR antagonist.

MATERIALS AND METHODS

Cell Lines and Cell Culture To generate AT,R-deficient mouse embryonic fibroblasts (MEFs), we isolated MEFs from 13.5-d-old embryos of AT,R deficient mice (The Jackson Laboratory, U.S.A.) with the C57BL/6 background, following the procedures as described previously. C57BL/6 MEFs and AT,R-deficient MEFs were infected with human 695-amino acid amyloid precursor protein (hAPP695) cDNA by a retrovirus-mediated method according to published methods.

The two kinds of cells were cultured in Dulbecco’s modified Eagle’s medium (DMEM) (Wako Pure Chemical Corporation, Osaka, Japan) supplemented with 10% fetal bovine serum (FBS) (Sigma-Aldrich, St. Louis, MO, U.S.A.). Cells were maintained at 37°C in an atmosphere of 5% CO2 in a tissue culture incubator. The hAPP695-infected fibroblasts were passaged with the same cell concentration for each pathway inhibitor administration.

Reagents ARB, Telm, and β-AR antagonists, propranolol (Prop) were purchased from Sigma-Aldrich dissolved in dimethyl sulfoxide (DMSO). β1-AR selective antagonists, atenolol (Aten), and bisoprolol (Biso), and β1-AR selective antagonist, ICI-118551 (ICI) were purchased from Sigma-Aldrich and dissolved in DMSO.

Aβ Enzyme-Linked Immunosorbent Assay (ELISA) Cells were plated in 10% FBS-DMEM (Wako Pure Chemical Corporation) and Aβ levels in the culture media were determined after 72h with ELISA kit purchased from Wako. All samples were measured in triplicate.

Preparation of Cell Lysate Cell lysate from fibroblasts expressing APP was prepared in RIPA buffer (50mM Tris–HCl (pH 7.5), 150mM NaCl, 1% NP-40, 0.5% sodium deoxycholate and 0.1% sodium lauryl sulfate) containing a protease inhibitor mixture. The protein concentration of the lysate was determined using bicinchoninic acid (BCA) protein assay kit (Thermo Fisher Scientific, Waltham, MA, U.S.A.).

Statistical Analysis All data are shown as the means ± standard error of the mean (S.E.M.). We compared group difference by one-way ANOVA followed by the post hoc Bonferroni-Dunn test for two or more groups against a control group. All data was analyzed by GraphPad Prism 5. p values <0.05 were considered statistically significant.

RESULTS

Effect of β-AR Antagonist, Prop on the Increase in Aβ Generation Caused by Telm Treatment Previously, we reported that Aβ generation is affected by β2-AR. To address this, we analyzed whether the increase in Aβ production caused by Telm treatment is affected by β2-AR antagonist.

Effect of Prop on the increase in Aβ generation following treatment with Telm. Fibroblasts overexpressing APP were cultured with Prop in the presence or absence of Telm for 72h. Aβ level in the cultured medium was measured with ELISA. Data were the average of four independent experiments. Error bars are means ± S.E.M. *p<0.05, ***p<0.005 by one-way ANOVA followed by a post hoc test. N.S., not significant.

β2-AR Antagonist Reduced an Increase in Aβ Generation Caused by the Treatment with Telm So, next clarified which, β-AR, β1- or β2-AR, is involved in the decrease in the stimulation of Aβ production by Telm. For this purpose, we examined the effect of β1-AR selective antagonists of Aten, and Biso, and the β2-AR selective antagonist, ICI on an increase in Aβ production by Telm. We found that ICI clearly inhibited Aβ production by Telm in a dose-dependent manner (Fig. 2A), while the treatment of Aten failed to inhibit an increase in Aβ production by Telm and Biso did not exhibit a dose-dependent inhibition of the stimulation of Aβ production by Telm. This result showed that an increase in Aβ production by Telm was selectively reduced by β2-AR antagonist, suggesting that the interaction between AT,R and β2-AR is involved in Telm-stimulation of Aβ production.

Biso appeared to slightly inhibit Telm-stimulation of Aβ production without a dose dependency. Since a small portion of Biso is known to antagonize β2-AR, the binding of β2-AR with Biso could diminish a part of Telm-stimulated Aβ generation by affecting the interaction of β2-AR and AT,R. Alternatively, β2-AR could be somehow involved in the Aβ generation pathway although the precise mechanism is not known.
The Effect of \( \beta_2 \)-AR Antagonist and Telm on \( \text{A}\beta\) Production in AT\(_1\)R-Deficient Cells

To further confirm that the interaction of AT\(_1\)R and \( \beta_2 \)-AR regulates \( \text{A}\beta\) generation, we next addressed whether \( \beta_2 \)-AR antagonist can influence \( \text{A}\beta\) generation in AT\(_1\)R deficient cells. As shown in Fig. 3A, the effect of Telm on \( \text{A}\beta\) generation in AT\(_1\)R deficient cells was not significantly inhibited by 20 \( \mu \text{M} \) of ICI or Prop, which is the same concentration with Telm, however, Telm-enhancing \( \text{A}\beta\) generation in wild type cells was inhibited by the treatment with ICI or Prop (Fig. 3B). Indeed, 10 \( \mu \text{M} \) ICI was sufficient to inhibit Telm-enhancing \( \text{A}\beta\) generation in wild type cells (Fig. 2A). Thus, it appears that a decrease in \( \text{A}\beta\) generation by the treatment with \( \beta_2 \)-AR, ICI or \( \beta \)-AR, Prop requires the presence of AT\(_1\)R, strongly suggesting that Telm stimulates \( \text{A}\beta\) generation through affecting the interaction of AT\(_1\)R and \( \beta_2 \)-AR. It is also found that \( \text{A}\beta 40\) generation is not changed by the treatment with Telm, but \( \text{A}\beta 42\) generation is slightly increased by the treatment with Telm, although the exact reason is not known at present (Fig. 3A). Unlike humans, mice have two AT\(_1\)R subtypes, AT\(_{1a}\) and AT\(_{1b}\), although AT\(_{1a}\) isoform predominates over the AT\(_{1b}\) in all tissues.\(^{17,18}\) Because the mice we used in this study is AT\(_{1a}\)-deficient mice (see “Materials and Methods”), a low level of AT\(_{1b}\) is likely to be expressed. Since ARB has a high affinity for both AT\(_1\)R subtypes,\(^{17}\) \( \text{A}\beta 42\) generation can be stimulated by Telm through AT\(_{1b}\).

**Fig. 2. \( \beta_2 \)-AR Antagonist Reduced the Increase in \( \text{A}\beta\) Generation Caused by Treatment with Telm**

A, Effect of \( \beta_2 \)-AR selective antagonist, ICI on the increase in \( \text{A}\beta\) production caused by Telm. Fibroblasts overexpressing APP were cultured with \( \beta_2 \)-AR selective antagonist, in the presence or absence of Telm for 72h. \( \text{A}\beta\) level in the cultured medium was measured with ELISA. Data are the average of four independent experiments. *\( p < 0.05 \), **\( p < 0.01 \), ***\( p < 0.005 \). N.S., not significant. B, Effect of \( \beta_2 \)-AR selective antagonists of Aten, and Biso on an increase in \( \text{A}\beta\) production caused by Telm. Fibroblasts overexpressing APP were cultured with \( \beta_2 \)-AR selective antagonists in the presence or absence of Telm for 72h. \( \text{A}\beta\) level in the cultured medium was measured with ELISA. Data were the average of four independent experiments. *\( p < 0.05 \). N.S., not significant.
Accumulating evidence demonstrates that GPCRs, classically considered to function as monomers, are actually organized as homodimers and heterodimerize with other GPCR family members. AT₁R, one of GPCRs, was also strongly suggested to be regulated in signal transduction by heterodimerization or crosstalk of AT₁R with β₂-AR.20,21) Because β₂-AR has the strongest binding affinity to AT₁R among the various ARBs, we hypothesize that this strong binding may trigger the similar signal transduction pathway to increase Aβ generation as angiotensin II does.3,4)

It is also shown that β₂-AR affects γ-secretase activity by regulating the formation of presenilin complex.5) However, it is not known whether the heterodimerization of AT₁R and β₂-AR is necessary for the regulation of γ-secretase activity. Therefore, in this study, we aimed to elucidate whether the heterodimerization or crosstalk of AT₁R with β₂-AR is crucial for γ-secretase activity. We found that the stimulation of Aβ production by Telm was inhibited by β₂-AR antagonist, while β₂-AR antagonists did not exhibit a significant inhibition. In addition, β₂-AR antagonist did not inhibit Aβ production in AT₁R-deficient cells. These results strongly suggested that the inhibitory effect of β₂-AR antagonist on Telm stimulation of Aβ production is dependent on the presence of AT₁R. Therefore, the stimulatory effect of Telm on Aβ production appears to result from the promotion of the interaction of AT₁R and β₂-AR. Taken together, the heterodimerization or crosstalk of AT₁R with β₂-AR is likely to increase Aβ production. β- Arrestin, which is known as one of the adaptor proteins, interacts with β₂-AR19) and the interaction of β₂-AR and AT₁R is suggested to promote the interaction of β- arrestin with β₂-AR.20,21) Because β-arrestin 2 is likely to regulate γ-secretase activity,22) the binding of Telm on AT₁R causes the conformational change of AT₁R that could promote the interaction of β-arrestin with β₂-AR, resulting in an increase in γ-secretase activity.

Since AT₁R and β₂-AR exist in the brain,23,24) our present study raised the following possibilities: (i) Aβ accumulation in the brain could be caused by the promotion of the heterodimerization or crosstalk of AT₁R and β₂-AR with aging, and (ii) some medicines targeting GPCR family members may affect the stimulation or inhibition of Aβ accumulation. Since the oxidation or glycation of membrane proteins or lipids is known to progress with aging,25,26) these changes, directly or indirectly, could cause the promotion of the heterodimerization or crosstalk of AT₁R and β₂-AR, which leads to the stimulation of Aβ production. It is also worth noting that there are many GPCR family members in the brain, including serotonin receptors, dopamine receptors and opioid receptors,27) and that there are many medicines targeting these GPCRs including inactivation of receptor-associated heterodimeric G protein and consequent downstream signaling. However, the precise mechanism underlying the regulation of the signal transduction by the heterodimers of GPCR family members remains to be elucidated, and also the GPCR family members forming the heterodimer need to be clarified.

Aβ is thought to be the cause of cognitive impairment of AD, and it is produced by the proteolytic cleavage of APP by β- and γ-secretase. Recently, we reported that AT₁R knockout in mice generates incomplete presenilin complex, resulting in a reduction of Aβ deposits in the brain. Therefore, AT₁R is involved in the formation of presenilin complex, regulating γ-secretase activity.3) Our previous study showed that one of ARBs, Telm, increased Aβ production via AT₁R, although AT₁R-deficient cells exhibited a reduction in Aβ generation. We found that Telm treatment stimulated the phosphorylation of Akt and increased Aβ generation, which was also found in angiotensin II treated cells, as previously shown.4) Because Telm has the strongest binding affinity to AT₁R among the various ARBs, we hypothesize that this strong binding may trigger the similar signal transduction pathway to increase Aβ generation as angiotensin II does.3,4)

Fig. 3. Deficiency of AT₁R Abolished the Effects of β₂-AR Antagonist and Telm on Aβ Production

A Agt⁻/⁻/APP⁺/⁻ cell

**p<0.01, ***p<0.005. N.S., not significant.

DISCUSSION

A, AT₁R-deficient fibroblasts overexpressing APP were cultured with ICI or Prop in the presence or absence of Telm for 72 h. Aβ level in the cultured medium was measured with ELISA. Data were the average of four independent experiments. Agt⁻/⁻/APP⁺/⁻ cells: AT₁R-deficient fibroblast cells overexpressing APP. B, wild-type fibroblasts overexpressing APP were cultured with ICI or Prop in the presence or absence of Telm for 72 h. Aβ level in the cultured medium was measured with ELISA. Data were the average of four independent experiments. Agt⁺//+/APP⁺ cells: wild-type fibroblasts expressing human APP. Error bars are means ± S.E.M. **p<0.01, ***p<0.005. N.S., not significant.
antidepressant and antipsychotic drugs. However, at present it is not known whether these medicines affect Aβ accumulation through modulating the heterodimerization or crosstalk of the target GPCR with AT1R or β2-AR.

Further study on the interaction of AT1R and β2-AR will be needed regarding the development of AD or the effect of the medicine targeting GPCR family members on Aβ accumulation.

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Conflict of Interest The authors declare no conflict of interest.

REFERENCES

1) Scheltens P, Blennow K, Breteler MM, de Strooper B, Frisoni GB, Salloway S, Van der Flier WM. Alzheimer’s disease. Lancet. 2018, 386, 505–515 (2016).
2) Selkoe DJ, Hardy J. The amyloid hypothesis of Alzheimer’s disease at 25 years. EMBO Mol. Med., 8, 595–608 (2016).
3) Liu J, Liu S, Matsumoto Y, Murakami S, Sugakawa Y, Kami A, Tanabe C, Maeda T, Michikawa M, Komano H, Zou K. Angiotensin type 1a receptor deficiency decreases amyloid β-protein generation and ameliorates brain amyloid pathology. Sci. Rep., 5, 12059 (2015).
4) Liu J, Liu S, Tanabe C, Maeda T, Zou K, Komano H. Differential effects of angiotensin II receptor blockers on Aβ generation. Neurosci. Lett., 567, 51–56 (2014).
5) Ni Y, Zhao X, Bao G, Zou L, Teng L, Wang Z, Song M, Xiong J, Bai Y, Pei G. Activation of β2-adrenergic receptor stimulates γ-secretase activity and accelerates amyloid plaque formation. Nat. Med., 12, 1390–1396 (2006).
6) Bussiere R, Lacampagne A, Reiken S, Liu X, Scheuerman V, Zalk R, Martin C, Checler F, Marks AR, Chami M. Amyloid β production is regulated by β2-adrenergic signaling-mediated post-translational modifications of the ymodian receptor. J. Biol. Chem., 292, 10153–10168 (2017).
7) Barki-Harrington L, Luttrell LM, Rockman HA. Dual inhibition of β-secretase and angiotensin II receptors by a single antagonist: a functional role for receptor-receptor interaction in vivo. Circulation, 108, 1611–1618 (2003).
8) Chun HJ, Ali ZA, Kojima Y, Kundu RK, Sheikh AY, Agrawal R, Zheng L, Leeper NJ, Pearl NE, Patterson AJ, Anderson JP, Tsao PS, Lenardo MJ, Ashley EA, Quertermous T. Apelin signaling antagonizes Ang II effects in mouse models of atherosclerosis. J. Clin. Invest., 118, 3343–3354 (2008).
9) AbdAlla S, Lother H, Langer A, el Faramawy Y, Quitterer U. Factor XIIIA transglutaminase crosslinks AT1 receptor dimers of monocytes at the onset of atherosclerosis. Cell, 119, 343–354 (2004).
10) Gudermann T, Nürnberg B, Schultz G. Receptors and G proteins as primary components of transmembrane signal transduction. Part 1. G-protein-coupled receptors: structure and function. J. Mol. Med. (Berl), 73, 51–63 (1995). http://www.ncbi.nlm.nih.gov/pubmed/7627630
11) Vassilatskik D, Hohmann JG, Zeng H, Li F, Ranchalis JE, Mortrud MT, Brown A, Rodriguez SS, Weller JR, Wright AC, Bergmann JE, Gaitanaris GA. The G protein-coupled receptor repertoires of human and mouse. Proc. Natl. Acad. Sci. U.S.A., 100, 4903–4908 (2003).
12) Goupil L, Laporte SA, Hébert TE. GPCR heterodimers: asymmetries in ligand binding and signalling output offer new targets for drug discovery. Br. J. Pharmacol., 168, 1101–1103 (2013).
13) Parmentier M. Heterodimer-specific signaling. Nat. Chem. Biol., 11, 244–245 (2015).
14) Thatthiah A, De Strooper B. The role of G protein-coupled receptors in the pathology of Alzheimer’s disease. Nat. Rev. Neurosci., 12, 73–87 (2011).
15) Komano H, Shiraishi H, Kawamura Y, Sai X, Suzuki R, Serneels L, Kawaichi M, Kitamura I, Yanagisawa K. A new functional screening system for identification of regulators for the generation of amyloid β-protein. J. Biol. Chem., 271, 39627–39633 (2002).
16) Baker JG. The selectivity of β-adrenoceptor antagonists at the human β1, β2 and β3 adrenoceptors. Br. J. Pharmacol., 144, 317–322 (2005).
17) Sasamura H, Hein L, Krieger JE, Pratt RE, Kobikia BK, Draz VJ. Cloning, characterization, and expression of two angiotensin receptor (AT-1) isoforms from the mouse genome. Biochem. Biophys. Res. Commun., 185, 253–259 (1992).
18) Draz VJ, Sasamura H, Hein L. Heterogeneity of angiotensin synthetic pathways and receptor subtypes: physiological and pharmacological implications. J. Hypertens. Suppl., 11, S13–S18 (1993). http://www.ncbi.nlm.nih.gov/pubmed/8315512
19) Shenoy SK, Lefkowitz RJ. Angiotensin II-Stimulated Signaling Through G Proteins and β-Adrenergic. Sci. STKE, 2005, em4 (2014).
20) Turu G, Szidonya L, Gáborik Z, Buday L, Spät A, Clark A,JL, Hunyady L. Differential β-arrestin binding of AT1 and AT2 angiotensin receptors. FEBS Lett., 580, 41–45 (2006).
21) Töth AD, Gyombolai P, Szlai B, Vármai P, Turu G, Hunyady L. Angiotensin type 1A receptor regulates β-arrestin binding of the β1-, β2- and β3-adrenergic receptor via heterodimerization. Mol. Cell. Endocrinol., 442, 113–124 (2017).
22) Thatthiah A, Horre K, Snellinx A, Vandeweyer E, Huang Y, Ciesielka M, De Kloe G, munch S, De Strooper B. β-Arrestin 2 regulates Aβ generation and γ-secretase activity in Alzheimer’s disease. Nat. Med., 19, 43–49 (2013).
23) Labandeira-García JL, Garrido-Gil P, Rodríguez-Pallares J, Valenzuela R, Borrajero A, Rodriguez-Perez AJ. Brain renin-angiotensin system and dopaminergic cell vulnerability. Front. Neuroanat., 8, 67 (2014).
24) Chai GS, Wang YY, Yasheng A, Zhao P. β2-Adrenergic receptor activation enhances neurogenesis in Alzheimer’s disease mice. Neuro Regen. Res., 11, 1617–1624 (2016).
25) Emerit J, Edeas M, Brouaire F. Neurodegenerative diseases and oxidative stress. Biomed. Pharmacother., 58, 39–46 (2004). http://www.ncbi.nlm.nih.gov/pubmed/14739060
26) Semb RD, Nicklett EJ, Ferrucci L. Does accumulation of advanced glycation end products contribute to the aging phenotype? J. Gerontol. A Biol. Sci. Med. Sci., 65A, 963–975 (2010).
27) Jacobson KA. New paradigms in GPCR drug discovery. Biochem. Pharmacol., 98, 541–555 (2015).