Intracerebroventricular Infusion of Angiotensin-(1–7) Ameliorates Cognitive Impairment and Memory Dysfunction in a Mouse Model of Alzheimer’s Disease

Ken Uekawa, Yu Hasegawa, Satoru Senju, Naomi Nakagata, Mingjie Ma, Takashi Nakagawa, Nobutaka Koibuchi and Shokei Kim-Mitsuyama

Departments of Pharmacology and Molecular Therapeutics, Kumamoto University Graduate School of Medical Sciences, Chuo-ku, Kumamoto, Japan

Department of Immunogenetics, Graduate School of Medical Sciences, Kumamoto University, Chuo-ku, Kumamoto, Japan

Division of Reproductive Engineering, Center for Animal Resources and Development, Kumamoto University, Chuo-ku, Kumamoto, Japan

Handling Associate Editor: Masahito Yamada

Accepted 20 March 2016

Abstract. This work was performed to test our hypothesis that angiotensin-(1–7) can ameliorate cognitive impairment and cerebrovascular reactivity in 5XFAD mice, a useful model of Alzheimer’s disease. 5XFAD mice received intracerebroventricular infusion of (1) vehicle, (2) angiotensin-(1–7), or (3) angiotensin-(1–7)+A779, a specific Mas receptor antagonist, for 4 weeks. Angiotensin-(1–7), through Mas receptor, significantly ameliorated cognitive impairment in 5XFAD mice. As estimated by acetazolamide-induced increase in cerebral blood flow, angiotensin-(1–7), through Mas receptor, enhanced cerebrovascular reactivity in 5XFAD mice. In conclusion, angiotensin-(1–7)/Mas receptor axis improves cognitive function and cerebrovascular function in a mouse model of Alzheimer’s disease.

Keywords: Acetazolamide, angiotensin-(1–7), cognitive function, Mas receptor, vascular reactivity, water maze test

INTRODUCTION

Emerging experimental, clinical, and epidemiological evidence supports the notion that the brain renin-angiotensin system (RAS) is potentially involved in the pathophysiology of Alzheimer’s disease (AD) and dementia [1–3]. Interestingly, treatment with RAS blockers of hypertensive patients is associated with slowing of cognitive impairment and AD progression [1, 4–8]. However, the exact mechanism underlying the contribution of RAS to AD is poorly understood.

It is established that the classical RAS consists of angiotensin-converting enzyme, angiotensin II, and angiotensin AT1 receptor and exerts deleterious effects on brain. Apart from the classical RAS,
The evolving concept of the RAS is that there exits protective arms of the RAS which exert protective effects against brain disease [3, 9–11]. Angiotensin-(1–7) is regarded as the major bioactive peptide of protective arms of the RAS [3, 9–11]. Recent experimental evidence indicates that angiotensin-(1–7) protects against brain injury such as ischemic stroke [9] as well as various cardiovascular diseases [10]. However, to our knowledge, there is no available information on the role of angiotensin-(1–7) in AD.

Therefore, we hypothesized that angiotensin-(1–7) can improve cognitive function in AD. To test our hypothesis, we examined the effect of angiotensin-(1–7) on cognitive function and cerebrovascular reactivity in AD mouse model.

MATERIALS AND METHODS

Animals

All experiments were approved by the Kumamoto University Committee for Laboratory Animal Care and Use. To obtain 5XFAD mice [12], cryopreserved embryos were purchased from Jackson Laboratory (Bar Harbor, Maine, USA) and implanted into pseudopregnant foster mice. 5XFAD mice were backcrossed into the C57BL/6J background at least 10 generations to reduce genetic variation. Animals were housed in a temperature-controlled (20 ± 2°C) and humidity-controlled (60%) room under a 12 h light/dark cycle (8:00/20:00).

Drugs

Ang-(1–7) was purchased from Peptide Institute, Inc. (Code: 4332-v; Osaka, Japan). A-779, a selective Mas receptor antagonist [13], was purchased from Bachem, Inc. (H-2888; Bachem, Switzerland). We prepared artificial cerebrospinal fluid (CSF) as a vehicle solution (composition in mmol/L: 150 Na, 3.0 K, 1.4 Ca, 0.8 Mg, 1.0 P, 155 Cl, equilibrated with 95%O2/5%CO2) according to the protocol of Alzet osmotic pumps, and the agents were dissolved in the artificial CSF.

Experimental protocol

Seventeen-month-old male 5XFAD mice were subjected to implantation of intracerebroventricular (ICV) infusion cannula and osmotic pump, as described below. These mice were randomly divided into three groups, and received continuous ICV infusion of (1) vehicle (artificial CSF), (2) Ang-(1–7) (500 ng/kg/h), or (3) Ang-(1–7) (500 ng/kg/h) plus A-779 (5.0 μg/kg/h) for 4 weeks. The dose of angiotensin-(1–7) was determined according to previous reports [14, 15].

Implantation of the ICV infusion cannula and osmotic pump

Implantation of infusion cannula into the cerebral ventricles and osmotic pump were performed on mice, according to previously reported method [16] with some modification. Briefly, mice were anesthetized with 1.5% isoflurane through a face mask and placed in a stereotactic apparatus. The scalp was reflected under sterile surgical conditions. Stainless steel cannulas (ALZET Brain Infusion Kit 3, DURECT Co., Cupertino, California, USA) were implanted in the left lateral ventricle, which were placed in relation to bregma 1.0 mm lateral and 0.5 mm posterior. The cannula were fixed on the skull and catheters were attached to extension tubes and linked to the ALZET osmotic pump (Model 1004, DURECT Co.), which were installed in subcutaneous pockets on the lateral back of the mice. Wounds were closed with nylon sutures.

Morris water maze test

To assess spatial learning and memory function, from 21 days to 26 days after the start of ICV infusion, the Morris water maze test was performed, according to our previous method [17] with a slight modification. Groups were blinded to the examiners. On the hidden platform test, the mice had 3 sessions at 20-min intervals per day on the following 5 consecutive days (day 1 to 5). On the probe test (day 6), the hidden platform was removed and the mice swam freely for 100 seconds. The number of times the mice cross the original platform location, time spent in quadrant, and distance traveled in quadrant were simultaneously recorded. The visible platform test was performed after the probe test on day 6.

Cerebral vascular reactivity to acetazolamide

Cerebral blood flow (CBF) was measured using a laser speckle blood flow imager (Omega Zone; Omegawave, Tokyo, Japan), as previously described [17]. The skull of each mouse was exposed by a midline scalp incision under 1.5–2.0% isoflurane.
and the ICV infusion cannula and osmotic pump were removed. Then, isoflurane was discontinued and anesthesia was maintained with urethane (700 mg/kg) and α-chloralose (60 mg/kg) intraperitoneally. CBF on the surface of the region of bilateral cerebral hemispheres was measured. To study the vasomotor reactivity to acetazolamide [18], three times of 10-second baseline CBF value was recorded and the mean value was calculated for determination of baseline CBF. Then, 100 mg/kg acetazolamide (Diamox; Sanwa Kagaku Kenkyusho co., Ltd, Nagoya, Japan) was injected intraperitoneally, and a 10-s CBF value was measured at 5 and 10 min after acetazolamide injection.

Immunohistochemistry of Aβ1–40 and Aβ1–42

Frozen brain tissue sections (8 μm thickness; 1.4–2.0 mm caudally from bregma) from mice sacrificed at 4 weeks of ICV infusion were incubated for 5 min in 90% formic acid and for 30 min in 0.3% H2O2/phosphate-buffered saline, and then stained with Aβ1–40 or Aβ1–42 antibody (1:100, Code No. 18580 and No. 18582, respectively; Immuno-Biological Laboratories Co., Ltd, Gunma, Japan), reacted with horseradish peroxidase-conjugated anti-rabbit IgG secondary antibody, and visualized with 3,3’-diaminobenzidine (Dako Cytomation). To evaluate the cerebral amyloid angiopathy, Aβ1–40 deposition was measured in two cortical branches of middle cerebral artery in each hemisphere (totally four cortical arteries) at x400 magnification using WinRoof Version 5.8 (Mitani Corporation, Fukui, Japan) and was expressed as the mean of % area of Aβ1–40 deposition in each mouse.

Measurement of soluble Aβ1–42 and Aβ oligomers

Fresh frozen cerebral hemispheres were homogenized in 5 volumes (w/v) of 1% Triton X-100 in Tris-buffered saline (TBS) (25 mM Tris and 137 mM NaCl, pH 7.6) containing protease inhibitors (protease inhibitor cocktail, 1 tablet in 50 ml solution; Roche). The homogenate was centrifuged at 100,000 g for 60 min at 4°C, and the supernatant was saved as the Triton X-100-soluble fraction [19]. The amounts of soluble Aβ1–42 in each fraction were quantified by ELISA assay kit (#27711, Immuno-Biological Laboratories Co., Ltd). Soluble Aβ oligomers were measured by ELISA kit (#27725, Immuno-Biological Laboratories Co., Ltd.).

Western blot analysis

Western blot analysis of brain tissue was performed according to our previous method [17]. Primary antibodies used were as follows: anti-GAPDH (x5000, Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA); anti-amyloid precursor protein (#MAB348)(x2000, Merck Millipore, MA, USA); anti-phospho-tau (#ab109390) (x50000, Abcam PLC, Cambridge, UK); anti-angiotensin-(1–7) Mas receptor (#AAR-013) (x2000, Alomone labs, Ltd., Jerusalem, Israel). Intensity of the bands was quantified by using analysis software (Image J; National Institute of Health, Bethesda, MD, USA). In individual samples, each value was corrected for GAPDH.

Statistical analysis

Values were expressed as the mean ± SEM. The difference among comparison groups was tested with appropriate statistical method, as shown in each Figure legend. p<0.05 was considered significant. The statistical analyses were performed with GraphPad Prism version 6.0 for Windows (GraphPad Software Inc., San Diego, CA, USA).

RESULTS

Cognitive function and vision

As shown by escape latency and cumulative escape latency of hidden test on Morris water maze test in Fig. 1A and B, ICV infusion of angiotensin-(1–7) significantly ameliorated cognitive impairment of 5XFAD mice (p<0.05), and this beneficial effect of angiotensin-(1–7) was significantly abolished by co-administration with A779 (p<0.05). As shown by the probe trial, angiotensin-(1–7) increased number of platform crossings in 5XFAD mice (p<0.05) and this effect was significantly abolished by A779 (p<0.05) (Fig. 1C). Time spent in quadrant and distance traveled in quadrant (Fig. 1D and E, respectively) were significantly greater in angiotensin-(1–7) group than vehicle and angiotensin-(1–7)+A779 group. On the other hand, there was no significant difference in visual acuity and any possible motor deficit among 3 groups of 5XFAD mice (Fig. 1F).

There was no significant difference in body weight, food intake, water intake, urine volume, blood pressure, and heart rate among 3 groups of 5XFAD mice (Supplementary Figure 1).
Cerebral vascular reactivity

As shown in Fig. 1G, angiotensin-(1–7) significantly augmented % increase of CBF by acetazolamide at 10 min \((p<0.05)\) and this augmentation was completely blocked by A779 co-administration \((p<0.05)\). ICV infusion of angiotensin-(1–7) tended to enhance AUC for 10 min of % increase of CBF in 5XFAD mice and A779 co-administration significantly reduced it \((p<0.05)\) (Fig. 1H).

Cerebral and cerebrovascular Aβ accumulation and soluble Aβ oligomers

As shown in Fig. 2A and B, ICV infusion of angiotensin-(1–7) tended to reduce % area of cerebrovascular Aβ deposition in 5XFAD mice and A779 co-administration significantly increased it compared with angiotensin-(1–7) alone \((p<0.05)\).

Angiotensin-(1–7) did not significantly affect % area of Aβ plaque in hippocampus and cortex (Fig. 2C and D, respectively), and cerebral soluble Aβ oligomers (Fig. 2E) of 5XFAD mice, regardless of A779 co-administration. Furthermore, soluble Aβ oligomers levels were comparable between all groups of 5XFAD mice (Fig. 2F).

Cerebral amyloid precursor protein, phospho-tau, and Mas receptor levels

Western blot analysis indicated that there was no significant difference in cerebral amyloid precursor...
Fig. 2. Effect of ICV infusion of angiotensin-(1–7) with or without A779 on % area of cerebrovascular Aβ deposition (B), amyloid-β plaque in hippocampus (C) and cortex (D), cerebral soluble Aβ (E), and soluble Aβ oligomers (F) of 5XFAD mice. Panel A indicates representative photomicrographs of Aβ1–40 deposition in cortical branch of middle cerebral artery (upper), hippocampal Aβ1–42 deposition (middle), and cortical Aβ1–42 deposition (lower) in 3 groups of 5XFAD mice. The purple stain is a nuclear staining with hematoxylin. In panel B, % area of vascular Aβ1–40 deposition was calculated by dividing positive area of vascular Aβ1–40 by total cross section area of the artery. Abbreviations used are the same as in Fig. 1. Values are means ± SEM. n = 11-12 in Ve, n = 11-12 in A(1–7), n = 5 in A(1–7)+A779. In panels B, C, D, E, and F, the statistical significance of differences was assessed with a one-way ANOVA followed by the Fisher’s exact test or Kruskal-Wallis followed by Dunn’s multiple comparison.

DISCUSSION

A growing body of evidence indicates that angiotensin-(1–7) and its receptor, the Mas receptor, are widely distributed in the central nervous system [20] and are potentially involved in the pathophysiology of brain diseases [9, 21–22]. However, to our knowledge, there is no report investigating the potential role of angiotensin-(1–7)/Mas receptor axis in AD. In the present work, we obtained the first evidence indicating that angiotensin-(1–7) ameliorates cognitive impairment in a mouse model of AD, through Mas receptor. Therefore, our present work provided a novel insight into the mechanism of cognitive dysfunction in AD as well as the role of renin-angiotensin system in brain disorder. 5XFAD mouse is characterized by the significant accumulation of Aβ plaque in cerebral vessels as well as neuronal cells and by the progression of cognitive impairment, and is regarded as a useful mouse model of AD [12, 23]. Moreover, we used old (17-month-old) 5XFAD mice with significant cognitive impairment, since the main purpose of this study was to examine the therapeutic effect of angiotensin-(1–7). As shown by water maze test, angiotensin-(1–7) ICV infusion, without affecting visual acuity and any possible motor deficit, significantly ameliorated cognitive impairment in 5XFAD mice, and these beneficial effects of angiotensin-(1–7) were abolished by co-administration of A779.
a specific Mas receptor blocker. On the other hand, chronic ICV infusion of angiotensin-(1–7) did not alter body weight, food or water intake, urine volume, blood pressure, or heart rate of 5XFAD mice, thereby showing that the beneficial effect of angiotensin-(1–7) on cognitive function was not associated with these parameters. These results provided the first evidence that angiotensin-(1–7), though Mas receptor, improved cognitive function in AD animal model, being opposite to the detrimental effect of central angiotensin II on AD animal [24].

Although AD is classified as a neurodegenerative disorder, accumulating evidence indicates that cerebral vascular disorder and disturbed CBF regulation are importantly involved in pathophysiology of AD [25–29]. Therefore, we also examined the effect of angiotensin-(1–7) on cerebrovascular reactivity to acetazolamide in 5XFAD mice. As shown by comparison of acetazolamide-induced increase in CBF among groups, angiotensin-(1–7) improved cerebral vascular reactivity in 5XFAD mice, and this improvement by angiotensin-(1–7) was blocked by co-administration of A779. Thus, angiotensin-(1–7)/Mas receptor axis seems to exert beneficial effect on cerebrovascular reactivity as well as cognitive function in AD mouse. However, the present study did not allow us to elucidate the possible association of cerebrovascular reactivity with cognitive function in 5XFAD mice.

Of note, quantitative analysis of cerebrovascular Aβ deposition suggested that angiotensin-(1–7)/Mas receptor axis might reduce cerebrovascular Aβ deposition in 5XFAD mice. In contrast to cerebrovascular Aβ deposition, hippocampal and cortical Aβ deposition or soluble Aβ was not apparently affected by angiotensin-(1–7) or A779, thereby providing no evidence for the involvement of angiotensin-(1–7)/Mas receptor axis in parenchymal Aβ deposition. CAA is thought to be involved in the progression of cognitive impairment in AD [25–30]. However, in the present study, we provided no data indicating the direct contribution of CAA to cognitive impairment in 5XFAD mice. Further study is required to elucidate the exact mechanism responsible for the amelioration of cognitive impairment by angiotensin-(1–7)/Mas receptor in AD.

In conclusion, we obtained the first evidence that angiotensin-(1–7)/Mas receptor axis improves cognitive function and cerebrovascular reactivity in AD mouse. Therefore, our work highlights angiotensin-(1–7)/Mas receptor axis as a novel promising therapeutic target of AD. However, further detailed study, including dose titration study and comparison of 5XFAD with wild-type mice, is needed to elucidate our proposal, since the beneficial effect of angiotensin-(1–7) at the dose used in this study was small rather than large.

ACKNOWLEDGMENTS

This work was partially supported by research grants from Astellas, Boehringer Ingelheim, Daiichi Sankyo, Takeda, Kyowa Hakko Kirin.

Authors’ disclosures available online (http://j-alz.com/manuscript-disclosures/15-0642r3).

SUPPLEMENTARY MATERIAL

The supplementary material is available in the electronic version of this article: http://dx.doi.org/10.3233/JAD-150642.

REFERENCES


