Biochemical and Biophysical Research Communications 424 (2012) 409–413

Contents lists available at SciVerse ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Deletion of microsomal prostaglandin E synthase-1 protects neuronal cells from cytotoxic effects of β-amyloid peptide fragment 31–35

Yukiko Kuroki [a], Yuka Sasaki [a], Daisuke Kamei [a,b], Yoshiharu Akitake [c,1], Mitsuo Takahashi [c,d], Satoshi Uematsu [e], Shizuo Akira [e], Yoshihito Nakatani [a], Ichiro Kudo [a,†], Shuntaro Hara [a,*]

[a] Department of Health Chemistry, School of Pharmacy, Showa University, Tokyo 142-8555, Japan
[b] Department of Research and Development for Innovative Medical Needs, School of Pharmacy, Showa University, Tokyo 142-8555, Japan
[c] Department of Clinical Pharmacology, Faculty of Pharmaceutical Sciences, Fukuoka University, Fukuoka 814-0180, Japan
[d] Choju Medical Institute, Fukushimura Hospital, Toyohashi, Aichi 441-8124, Japan
[e] Department of Host Defense, Research Institute for Microbial Diseases, Osaka University, Osaka 565-0871, Japan

ARTICLE INFO

Article history:
Received 15 June 2012
Available online 2 July 2012

Keywords:
mPGES-1
Prostaglandin E_2
β-Amyloid
Neurotoxicity
Alzheimer's disease

ABSTRACT

Epidemiological studies have suggested that the long-term use of nonsteroidal anti-inflammatory drugs that inhibit cyclooxygenase (COX) activity moderates the onset or progression of Alzheimer's disease (AD). Thus it has been suggested that prostaglandin E_2 (PGE_2), a major end-product of COX, may play a pathogenic role in AD, but the involvement of PGE synthase (PGES), a terminal enzyme downstream from COX, has not been fully elucidated. To examine the involvement in AD pathology of microsomal PGES-1 (mPGES-1), a PGES enzyme, we here prepared primary cerebral neuronal cells from the cerebri of wild-type and mPGES-1-deficient mice and then treated them with β-amyloid (Aβ) fragment 31–35 ($A\beta_{31-35}$), which represents the shortest sequence of native Aβ peptide required for neurotoxicity. Treatment of wild-type neuronal cells with $A\beta_{31-35}$ induced mPGES-1 gene expression and PGE_2 production, followed by significant apoptotic cell death, but apoptosis was not induced in mPGES-1-deficient cells. Furthermore, the combined treatment of $A\beta_{31-35}$ and PGE_2 induced apoptosis in mPGES-1-deficient neuronal cells. These results indicated that mPGES-1 is induced during Aβ-mediated neuronal cell death and is involved in Aβ-induced neurotoxicity associated with AD pathology.

1. Introduction

Alzheimer's disease (AD) is the most common neurodegenerative disorder of the elderly and is characterized by senile plaques in several regions of the central nervous system [1]. A number of epidemiological studies have shown that the long-term use of nonsteroidal anti-inflammatory drugs (NSAIDs) reduces the risk of developing AD and delays its onset [2,3]. It is known that NSAIDs inhibit the activity of cyclooxygenase (COX), which catalyzes the conversion of arachidonic acid to prostaglandin H_2 (PGH_2), the precursor of PGE_2 and other PGs. Of the two COX isoforms, COX-1 is expressed constitutively in most tissues and is generally responsible for the production of the PGs that control normal physiological functions, while COX-2 is induced in response to mitogens, cytokines, and cellular transformation [4]. Although it remains unclear precisely how NSAIDs inhibit AD development, several studies have shown that elevated COX-2 expression is present in the cerebral cortex of the AD brain and that expression levels are correlated with amyloid plaque density and neurofibrillary tangles [5–7]. Levels of PGE_2, the main product of COX, have been found to be significantly elevated in the cerebrospinal fluid of AD patients [8], thus suggesting that PGE_2 signaling may function in AD development.

PGE_2 is synthesized from COX-derived PGH_2 by PGE synthase (PGES) activity. Thus far, three PGES enzymes have been identified: microsomal PGES (mPGES)-1, mPGES-2, and cytosolic PGES (cPGES) [9]. Among these PGES isozymes, mPGES-1 is induced by proinflammatory stimuli and is downregulated by anti-inflammatory glucocorticoids as in the case of COX-2, and is functionally coupled with COX-2 in marked preference to COX-1 [9,10]. The induction of mPGES-1 expression has been observed in various

Abbreviations: Aβ, β-amyloid peptide; $A\beta_{31-35}$, Aβ fragment 31–35; AD, Alzheimer's disease; COX, cyclooxygenase; cPGES, cytosolic PGES; GFAP, glial fibrillary acidic protein; MAP-2, microtubule-associated protein-2; mPGES, microsomal PGES; NSAID, nonsteroidal anti-inflammatory drug; PG, prostaglandin; PGES, PGE synthase.

* Corresponding author at: Department of Health Chemistry, School of Pharmacy, Showa University, 1-5-8 Hatanodai, Shinagawa-ku, Tokyo 142-8555, Japan. Fax: +81 3 3784 8245.
E-mail address: haras@pharm.showa-u.ac.jp (S. Hara).
[1] Present address: Department of Regenerative Medicine and Tissue Engineering, National Cerebral and Cardiovascular Center Research Institute, Osaka, Japan.
[†] Deceased.

0006-291X/$ - see front matter © 2012 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.bbrc.2012.06.121

conditions and processes in which COX-2-driven PGE_2 has been implicated, including rheumatoid arthritis, the febrile response, and reproduction [9]. It has also been reported that mPGES-1 levels were elevated in Western Blots of middle frontal gyrus tissue extracts from AD patients relative to those of age-matched controls [11]. Furthermore, Satoh et al. reported that β-amyloid peptide (Aβ), a major protein component of brain senile plaques in AD, induced mPGES-1 expression in rat astrocytes *in vitro* [12]. However, little is known about the involvement of mPGES-1, a downstream enzyme of COX-2, in the pathophysiology of AD.

Aβ has been shown to play a central role in AD development. It is well established that Aβ possesses neurotoxic activity [13]. Aβ neurotoxicity has been associated with peptide self-aggregation, which leads to the formation of amyloid-like fibrils and eventually to neuronal cell death through apoptosis [14]. Several small Aβ fragments induce cytotoxic and pro-apoptotic effects similar to those observed for native Aβ, $Aβ_{1-42}$ and $Aβ_{1-40}$, although it does not exhibit any aggregation behavior [15]. Among them, Aβ fragment 31–35 ($Aβ_{31-35}$) represents the shortest sequence of native peptides required for cytotoxicity and has been used to evaluate the initial events in Aβ-mediated neurotoxicity-associated AD pathology [16–19]. In the present study, to examine the involvement of mPGES-1 in Aβ-induced neurotoxicity, we prepared primary cerebral neuronal cells from the cerebri of wild-type and mPGES-1-deficient mice and then treated them with $Aβ_{31-35}$. The results showed that mPGES-1 is induced in neuronal cells by $Aβ_{31-35}$ and subsequently involved in $Aβ_{31-35}$-mediated apoptotic neuronal cell death.

2. Materials and methods

2.1. Primary culture of cerebral neuronal cells

Mouse studies were approved by the Institutional Animal Care and Use Committees of Showa University. mPGES-$1^{-/-}$ mice were established as described previously [20,21], and backcrossed at least ten generations with BALB/c mice [22].

Primary cerebral neuronal cells were prepared from the cerebri of 16.5-day-old embryos of wild-type mice and those of mPGES-$1^{-/-}$ mice using the Nerve-Cell Culture System (Sumitomo Bakelite, Tokyo, Japan). In brief, cerebral tissues were cleaned of meninges, minced, and treated with a protease mixture. After mechanical dissociation by pipetting and treatment with dissociation solution, we resuspended the cells in nerve cell culture medium, and then plated them onto poly-L-lysine-coated plates or glass dishes (Φ35 mm) at a density of 2.4×10^6 cells/well. After 7 days of culture, neuronal cells were examined for the effects of $Aβ_{31-35}$ (Peptide Institute, Inc., Minoh, Japan) on the expression of mPGES-1 and the cell death. Viable cells were peeled off by pipetting, and were counted using the trypan blue exclusion method. Neuronal cell identity was confirmed by immunostaining with the neuron marker anti-microtubule-associated protein-2 (MAP-2) (Sigma, St. Louis, MO, USA) and astrocyte marker anti-glial fibrillary acidic protein (GFAP) antibodies (Chemicon, Billerica, MA, USA). The amount of PGE_2 was measured using enzyme immunoassay kits (Cayman Chemical, Ann Arbor, MI, USA).

2.2. RT-PCR analysis

Total RNA was extracted from mouse primary cerebral neuronal cells using Trizol Reagent (Invitrogen, Carlsbad, CA, USA), and RT-PCR was performed using SuperScript III Reverse Transcriptase (Invitrogen) and Ex-Taq Polymerase (TaKaRa Bio, Inc., Otsu, Japan). Synthesis of cDNA was performed with 2 μg of the total RNA isolated from mouse primary cerebral neuronal cells, oligo dT primers, and SuperScript III Reverse Transcriptase (Invitrogen). Subsequent amplifications of the partial cDNA fragments were performed by Ex-Taq Polymerase using the reverse-transcribed products as a template with a set of specific oligonucleotide primers as follows: mPGES-1 sense 5′-GGCTGTCATCACAGGCCAGATGAGG-3′ and antisense 5′-CAGGAGAACTGGGCCAGGACATAGG-3′; COX-2 sense 5′-GGTCTGGTGCCTGGTCTGATGATG-3′ and antisense 5′-GTCCTTTCAAGGAGAATGGTGC-3′; and GAPDH sense 5′-TCGTGGAT CTGACGTGCCGCCTG-3′ and antisense 5′-CACCACCCTGTTGCTGTA GCCGTAT. The PCR mixtures were subjected to 35 (for mPGES-1 and COX-2) or 25 (for GAPDH) cycles of amplification by denaturation (45 s at 94 ℃), annealing (60 s at 60 ℃) and elongation (60 s at 72 ℃). The PCR products were analyzed by 1% agarose gel electrophoresis with ethidium bromide.

2.3. TUNEL staining experiments

The TUNEL staining experiments were performed as previously described [23]. Briefly, after treatment with 30 μM $Aβ_{31-35}$ and/or 25 μM PGE_2, the cells were fixed for 25 min in 4% paraformaldehyde in phosphate-buffered saline, permeabilized for 5 min in 0.2% Triton X-100, and then stained using the DeadEnd Fluorometric TUNEL system (Promega, Madison, WI, USA) according to the manufacturer's instructions. For the positive control experiments, the cells were treated with DNase I (TaKaRa Bio, Inc.) for 10 min before the staining. Three randomly chosen microscopic fields were captured in images, and the number of TUNEL-positive neuronal nuclei was calculated.

3. Results

3.1. Induction of mPGES-1 expression in $Aβ_{31-35}$-treated neuronal cells in vitro

Primary cerebral neuronal cells were prepared from the cerebri of 16.5-day-old mouse embryos and were then treated with $Aβ_{31-35}$. As shown in Fig. 1A, RT-PCR analysis revealed that mPGES-1 mRNA levels markedly increased with $Aβ_{31-35}$ treatment, without altering COX-2 expression. Increased production of PGE_2 was also observed, together with an increase in mPGES-1 expression 48 h after $Aβ_{31-35}$ treatment (Fig. 1C). On the other hand, neither an induction of mPGES-1 nor an increment in PGE_2 production was observed in $Aβ_{31-35}$-treated neuronal cells derived from mPGES-1-deficient mice. These results indicated that mPGES-1 contributes to the formation of PGE_2 in $Aβ_{31-35}$-treated neuronal cells.

Furthermore, to determine the cell type that expresses mPGES-1, we carried out double-immunostaining analyses of mPGES-1 and GFAP or MAP-2. We found that the mPGES-1-immunoreactive signals were enhanced near the nuclear membrane after $Aβ_{31-35}$ treatment (Fig. 1B). These signals were observed in GFAP-positive astrocytes as well as in some MAP-2-positive neurons.

3.2. mPGES-1 deficiency ameliorates $Aβ_{31-35}$-induced neuronal cell death in vitro

We then examined the effects of mPGES-1 deficiency on $Aβ_{31-35}$-induced neuronal cell death *in vitro*. The number of primary neuronal cells derived from wild-type mice increased during the study period (Fig. 2A). After 48 h of $Aβ_{31-35}$ treatment, this increase was significantly repressed. Double-immunostaining of GFAP and MAP-2 revealed that both GFAP-positive astrocytes and MAP-2-positive neurons proliferated during the observation period and the growth of each was suppressed by $Aβ_{31-35}$ (Fig. 2B). On the other hand, $Aβ_{31-35}$ treatment did not suppress the proliferation of

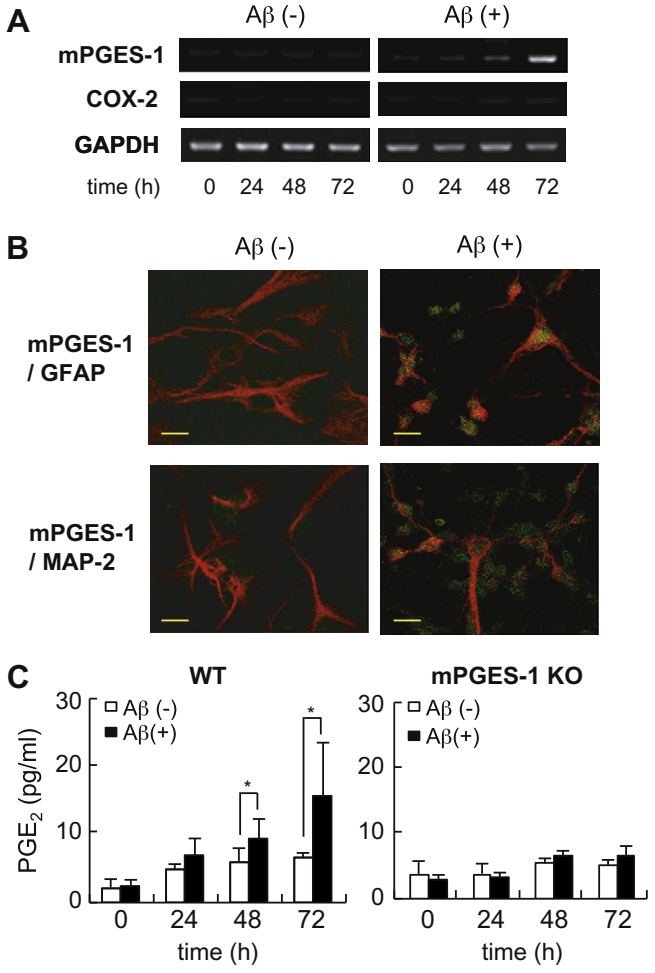

Fig. 1. Induction of mPGES-1 expression and PGE_2 production in $A\beta_{31-35}$-treated mouse cerebri neuronal cells. (A) RT-PCR analysis for mPGES-1 expression in neuronal cells. RNA was prepared from neuronal cells cultured with or without 30 μM $A\beta_{31-35}$ for the indicated times, and then RT-PCR analyses for mPGES-1, COX-2 and GAPDH (as a control) were performed. Representative results of at least three experiments are shown. (B) Immunocytostaining analysis of mPGES-1 expression in neuronal cells. mPGES-1 (green) and GFAP or MAP-2 (red) were double-immunostained in neuronal cells cultured with or without 30 μM $A\beta_{31-35}$ for 72 h. Bar: 20 μm. (C) PGE_2 production from neuronal cells. Neuronal cells were prepared from wild-type (left) and mPGES-1 KO mice (right), and then were cultured with (closed columns) or without 30 μM $A\beta_{31-35}$ (open columns) for the indicated times. Amounts of PGE_2 in culture medium from the cultured neuronal cells were assayed used an enzyme immunoassay kit. All values are means ± SD for at least 4 independent experiments. $^*P < 0.05$ vs non-treated cells.

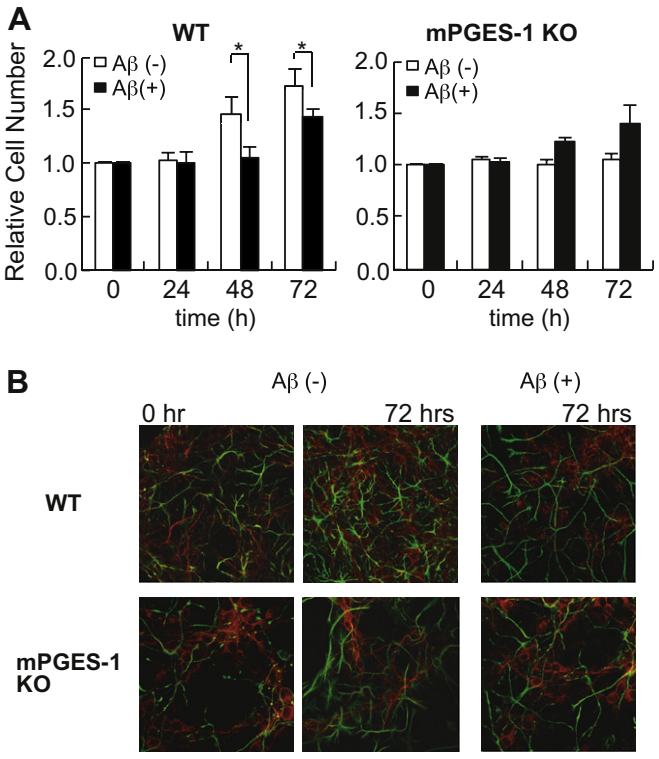

Fig. 2. Effects of mPGES-1 deficiency on $A\beta_{31-35}$-induced cell death of mouse cerebri neuronal cells. (A) Neuronal cells prepared from wild-type (left) and mPGES-1 KO mice (right) were cultured with (closed columns) or without 30 μM $A\beta_{31-35}$ (open columns) for the indicated times, and the cell numbers were counted. All values are means ± SD for at least 3 independent experiments. $^*P < 0.05$ vs non-treated cells. (B) GFAP (green) and MAP-2 (red) were double immunostained in wild-type mouse- (upper) or mPGES-1 KO mouse-derived (lower) neuronal cells cultured with or without 30 μM $A\beta_{31-35}$ for 72 h.

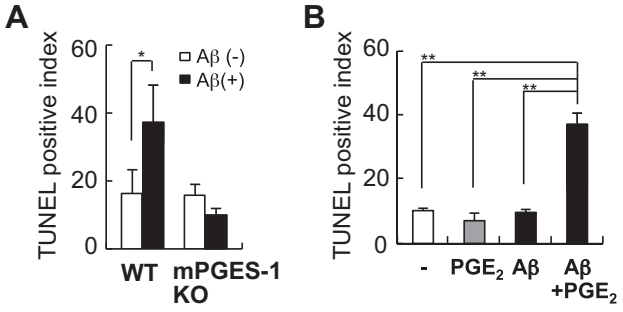

Fig. 3. Effects of mPGES-1 deficiency on $A\beta_{31-35}$-induced apoptosis in mouse cerebri neuronal cells. (A) Wild-type mouse- (left) or mPGES-1 KO mouse-derived (right) neuronal cells cultured with (closed columns) or without 30 μM $A\beta_{31-35}$ (open columns) for 72 h, and the apoptotic cells were subjected to TUNEL staining. All values are means ± SD for at least 5 independent experiments. $^*P < 0.05$ vs non-treated cells. (B) mPGES-1 KO mouse-derived neuronal cells were cultured with or without 30 μM $A\beta_{31-35}$ and 25 μM PGE_2 for 72 h, and the apoptotic cells were subjected to TUNEL staining. All values are means ± SD for at least 3 independent experiments. $^{**}P < 0.01$ vs $A\beta_{31-35}$ and PGE_2-treated cells.

neuronal cells derived from mPGES-1-deficient mice. Conversely, $A\beta_{31-35}$ modestly increased the growth of mPGES-1-deficient cells (Fig. 2A).

As it had been shown that $A\beta_{31-35}$ induces apoptosis in cultured neuronal cells [16–19], we next carried out a TUNEL staining analysis to examine the effects of an mPGES-1 deficiency on $A\beta_{31-35}$-induced apoptosis. As shown in Fig. 3A, the number of TUNEL-positive apoptotic cells increased when WT neuronal cells were treated with $A\beta_{31-35}$ for 72 h. On the other hand, apoptosis was not induced by $A\beta_{31-35}$ treatment of mPGES-1-deficient neuronal cells. Moreover, PGE_2 was not as effective as $A\beta_{31-35}$ at inducing apoptosis in mPGES-1-deficient cells; however, the combined treatment of $A\beta_{31-35}$ and PGE_2 did induce apoptosis in these cells (Fig. 3B). These results indicated that $A\beta_{31-35}$-induced mPGES-1-dependent PGE_2 production is necessary, but not sufficient, for $A\beta_{31-35}$-induced neuronal cell apoptosis.

4. Discussion

In the present study, we demonstrated that mPGES-1 is induced in $A\beta_{31-35}$-treated mouse cerebri nerve cells (Fig. 1) and that mPGES-1 deficiency ameliorates $A\beta_{31-35}$-induced apoptosis of these cells *in vitro* (Figs. 2 and 3). $A\beta_{31-35}$ has been used to evaluate

the initial events in Aβ-mediated neurotoxicity-associated AD pathology [16–19]. These results indicated that the induction of mPGES-1 is involved in Aβ-induced neurotoxicity associated with AD pathology.

As shown in Fig. 1A, $A\beta_{31-35}$ markedly increased mPGES-1 mRNA without eliciting any alterations in COX-2 expression in mouse neuronal cells. The increase in mPGES-1 expression is strongly correlated with the induction of COX-2 in many pathological conditions, and mPGES-1 has been shown to be preferentially coupled with COX-2 activity to increase the pathologic production of PGE_2 [9,10]. However, such findings do not reflect what is always the case. Sandee et al. reported that mPGES-1 was overexpressed and involved in PGE_2 production in both COX-1 and COX-2 deficient cells [24]. Several studies have shown that COX-2 expression is elevated in the cerebral cortex of the AD brain [5–7]; however, it should be noted that decreased expression of COX-2 in end-stage AD has also been reported [25]. mPGES-1 may play more a critical role than COX-2 in pathogenic PGE_2 production involved in AD pathology.

Our results also indicated that mPGES-1-derived PGE_2 might be involved in $A\beta_{31-35}$-induced apoptosis of neuronal cells *in vitro* (Figs. 2 and 3). PGE_2 exerts its biological actions via binding to four specific receptor subtypes known as EP1, EP2, EP3 and EP4 [26]. Studies using EP2-deficient mice have shown that among these four EPs, an EP present on microglia, EP2, plays an important role in Aβ-activated neurotoxicity [27,28]. Shie et al. used primary cultures of wild-type neurons and microglia from either wild-type or EP2-deficient mice, and found that Aβ-treated wild-type microglia enhanced Aβ-induced apoptosis in wild-type neurons, but that Aβ-treated EP2-deficient microglia did not have the same effect [27]. Aβ-induced expression of inducible nitric oxide synthase, which can cause neurotoxicity, was also reduced in EP2-deficient microglia. Furthermore, Liang et al. showed that a deletion of EP2 in APP-swe-PS1ΔE9 mice, an AD model mouse strain, resulted in a marked reduction in age-dependent lipid peroxidation in brain tissues [28]. Taken together, the results of these two reports indicated that the activation of EP2 by PGE_2 leads to the increased production of microglial superoxides and neurotoxins, and subsequently of reactive oxygen species, which in turn promotes lipid peroxidation and injury in neurons. We also found that a deletion of mPGES-1 partially inhibited the $A\beta_{31-35}$-induced generation of 8-isoprostane, a lipid peroxide, in a mixed culture of neurons, astrocytes and microglia (data not shown). mPGES-1-derived PGE_2 produced in response to Aβby astrocytes or neurons might act on microglial EP2 and promote oxidative damage leading to neuronal cell death.

Previous studies have demonstrated that PGE_2-EP2 signaling shows bidirectional effects that enhance Aβ-induced neuronal cell death and protect neuronal cells against Aβ-induced cell death [29]. Although PGE_2 and EP2 agonists have neurotoxic effects [30], lower concentrations of PGE_2 and EP2 or EP4 agonists were neuroprotective against Aβ toxicity [29]. It was noteworthy in the present study that mPGES-1-deficient neuronal cells did not grow in the absence of $A\beta_{31-35}$ during the entire study period (Fig. 2). These results suggest that a small amount of mPGES-1-derived PGE_2 might be involved in neuronal cell maintenance and growth. We also found that $A\beta_{31-35}$ increased rather than suppressed the growth of mPGES-1-deficient cells. It has been reported that Aβ induces arachidonic acid release by the activation of phospholipase A_2 in primary neuronal cells [31]. In mPGES-1-deficient cells, $A\beta_{31-35}$ might enhance certain arachidonate metabolites other than PGE_2, that stimulate neuronal cell growth. Further studies are still needed to reveal the mechanism underlying $A\beta_{31-35}$-enhanced growth of mPGES-1-deficient neuronal cells.

In conclusion, our results indicated that mPGES-1 is induced by Aβ and subsequently plays a critical role in Aβ-mediated apoptotic neuronal cell death associated with AD pathology. Although epidemiological studies have suggested that long-term use of NSAIDs moderates the onset or progression of AD, their intake is frequently associated with gastrointestinal side effects. Because the COX expressed in the gastrointestinal mucosa is primarily COX-1, in recent years efforts have focused on the development of highly selective COX-2 inhibitors with an improved gastric tolerability profile. However, severe cardiovascular adverse reactions challenged the initial enthusiasm for this new class of anti-inflammatory drugs. Blockage of mPGES-1 could form the basis for a novel therapeutic strategy for treating AD, and may represent a safer approach than standard therapies involving NSAIDs.

Acknowledgments

This work was supported in part by Grants-in-Aid for Comprehensive Research on Aging and Health, and Research on Dementia from the Ministry of Health, Labor and Welfare of Japan, and Grants-in-Aid for Scientific Research, Private University High Technology Research Center Project matching fund subsidy, and MEXT-Supported Program for the Strategic Research Foundation at Private Universities from the Ministry of Education, Science, Culture, Sports and Technology of Japan.

References

[1] D.J. Selkoe, Alzheimer's disease: genes, proteins, and therapy, Physiol. Rev. 81 (2001) 741–766.
[2] K.P. Townsend, D. Praticò, Novel therapeutic opportunities for Alzheimer's disease: focus on nonsteroidal anti-inflammatory drugs, FASEB J. 19 (2005) 1592–1601.
[3] P.L. McGeer, E.G. McGeer, NSAIDs and Alzheimer disease: epidemiological, animal model and clinical studies, Neurobiol. Aging 28 (2007) 639–647.
[4] W.L. Smith, E.A. Meade, D.L. DeWitt, Interactions of PGH synthase isozymes-1 and -2 with NSAIDs, Ann. N. Y. Acad. Sci. 744 (1994) 50–57.
[5] G.M. Pasinetti, P.S. Aisen, Cyclooxygenase-2 expression is increased in frontal cortex of Alzheimer's disease, Neuroscience 87 (1998) 319–324.
[6] L. Ho, C. Pieroni, D. Winger, D.P. Purohit, P.S. Aisen, G.M. Pasinetti, Regional distribution of cyclooxygenase-2 in the hippocampal formation in Alzheimer's disease, J. Neurosci. Res. 57 (1999) 295–303.
[7] K. Fujimi, K. Noda, K. Sasaki, Y. Wakisaka, Y. Tanizaki, M. Iida, Y. Kiyohara, S. Kanba, T. Iwaki, Altered expression of COX-2 in subdivisions of the hippocampus during aging and in Alzheimer's disease: the Hisayama study, Dement. Geriatr. Cogn. Disord. 23 (2007) 423–431.
[8] T.J. Montine, K.R. Sidell, B.C. Crews, W.R. Markesbery, L.J. Marnett, L.J. Roberts II, J.D. Morrow, Elevated CSF prostaglandin E_2 levels in patients with probable AD, Neurology 53 (1999) 1495–1498.
[9] S. Hara, D. Kamei, Y. Sasaki, A. Tanemoto, Y. Nakatani, M. Murakami, Prostaglandin E synthases: Understanding their pathophysiological roles through mouse genetic models, Biochimie 92 (2010) 651–659.
[10] M. Murakami, H. Naraba, T. Tanioka, N. Semmyo, Y. Nakatani, F. Kojima, T. Ikeda, M. Fueki, A. Ueno, S. Oh-ishi, I. Kudo, Regulation of prostaglandin E_2 biosynthesis by inducible membrane-associated prostaglandin E_2 synthase that acts in concert with cyclooxygenase-2, J. Biol. Chem. 275 (2000) 32783–32792.
[11] U.A. Chaudhry, H. Zhuang, B.J. Crain, S. Doré, Elevated microsomal prostaglandin-E synthase-1 in Alzheimer's disease, Alzheimers Dement. 4 (2008) 6–13.
[12] K. Satoh, Y. Nagano, C. Shimomura, N. Suzuki, Y. Saeki, H. Yokota, Expression of prostaglandin E synthase mRNA is induced in beta-amyloid treated astrocytes, Neurosci. Lett. 283 (2000) 221–223.
[13] B.A. Yankner, L.K. Duffy, D.A. Kirschner, Neurotrophic and neurotoxic effects of amyloid beta protein: reversal by tachykinin neuropeptides, Science 250 (1990) 279–282.
[14] C.W. Cotman, C.J. Pike, A. Copani, β-Amyloid neurotoxicity: a discussion of in vitro findings, Neurobiol. Aging 13 (1992) 587–590.
[15] L.L. Iversen, R.J. Mortishire-Smith, S.J. Pollack, M.S. Shearman, The toxicity *in vitro* of β-amyloid protein, Biochem. J. 311 (1995) 1–16.
[16] X.Z. Yan, J.T. Qiano, Y. Dou, Z.D. Qiao, β-amyloid peptide fragment 31–35 induces apoptosis in cultured cortical neurons, Neuroscience 92 (1999) 177–184.
[17] J.S. Qi, J.T. Qiao, Amyloid β-protein fragment 31–35 forms ion channels in membrane patches excised from rat hippocampal neurons, Neuroscience 105 (2001) 845–852.
[18] F. Misiti, G.E. Martorana, G. Nocca, E. Di Stasio, B. Giardina, M.E. Clementi, Methionine 35 oxidation reduces toxic and pro-apoptotic effects of the amyloid β-protein fragment (31–35) on isolated brain mitochondria, Neuroscience 126 (2004) 297–303.

[19] F. Misiti, B. Sampaolese, M. Pezzotti, S. Marini, M. Coletta, L. Ceccarelli, B. Giardina, M.E. Clementi, Aβ(31–35) peptide induce apoptosis in PC 12 cells: contrast with Aβ(25–35) peptide and examination of underlying mechanisms, Neurochem. Int. 46 (2005) 575–583.
[20] D. Kamei, K. Yamakawa, Y. Takegoshi, M. Mikami-Nakanishi, Y. Nakatani, S. Oh-Ishi, H. Yasui, Y. Azuma, N. Hirasawa, K. Ohuchi, H. Kawaguchi, Y. Ishikawa, T. Ishii, S. Uematsu, S. Akira, M. Murakami, I. Kudo, Reduced pain hypersensitivity and inflammation in mice lacking microsomal prostaglandin E synthase-1, J. Biol. Chem. 279 (2004) 33684–33695.
[21] D. Kamei, M. Murakami, Y. Sasaki, Y. Nakatani, M. Majima, Y. Ishikawa, T. Ishii, S. Uematsu, S. Akira, S. Hara, I. Kudo, Microsomal prostaglandin E synthase-1 in both cancer cells and hosts contributes to tumor growth, invasion and metastasis, Biochem. J. 425 (2010) 361–371.
[22] Y. Sasaki, D. Kamei, Y. Ishikawa, T. Ishii, S. Uematsu, S. Akira, M. Murakami, S. Hara, Microsomal prostaglandin E synthase-1 is involved in multiple steps of colon carcinogenesis, Oncogene 31 (2012) 2943–2952.
[23] H. Zhang, Q. Li, R.K. Graham, E. Slow, M.R. Hayden, I. Bezprozvanny, Full length mutant huntingtin is required for altered Ca^{2+} signaling and apoptosis of striatal neurons in the YAC mouse model of Huntington's disease, Neurobiol. Dis. 31 (2008) 80–88.
[24] D. Sandee, S. Sivanuntakorn, V. Vichai, J. Kramyu, K. Kirtikara, Up-regulation of microsomal prostaglandin E synthase-1 in COX-1 and COX-2 knock-out mouse fibroblast cell lines, Prostaglandins Other Lipid Mediat. 88 (2009) 111–116.
[25] A.V. Yermakova, M.K. O'Banion, Downregulation of neuronal cyclooxygenase-2 expression in end stage Alzheimer's disease, Neurobiol. Aging 22 (2001) 823–836.
[26] Y. Sugimoto, S. Narumiya, Prostaglandin E receptors, J. Biol. Chem. 282 (2007) 11613–11617.
[27] F.S. Shie, R.M. Breyer, T.J. Montine, Microglia lacking E prostanoid receptor subtype 2 have enhanced Aβ phagocytosis yet lack Aβ-activated neurotoxicity, Am. J. Pathol. 166 (2005) 1163–1172.
[28] X. Liang, Q. Wang, T. Hand, L. Wu, R.M. Breyer, T.J. Montine, K. Andreasson, Deletion of the prostaglandin E_2 EP2 receptor reduces oxidative damage and amyloid burden in a model of Alzheimer's disease, J. Neurosci. 25 (2005) 10180–10187.
[29] V. Echeverria, A. Clerman, S. Doré, Stimulation of PGE_2 receptors EP2 and EP4 protects cultured neurons againsat oxidative stress and cell death following β-amyloid exposure, Eur. J. Neurosci. 22 (2005) 2199–2206.
[30] T. Takadera, Y. Shiraishi, T. Ohyashiki, Prostaglandin E_2 induced caspase-dependent apoptosis possibly through activation of EP2 receptors in cultured hippocampal neurons, Neurochem. Lett. 45 (2004) 713–719.
[31] R.O. Sanchez-Mejia, J.W. Newman, S. Toh, G.Q. Yu, Y. Zhou, B. Halabisky, M. Cissé, K. Scearce-Levie, I.H. Cheng, L. Gan, J.J. Palop, J.V. Bonventre, L. Mucke, Phospholipase A_2 reduction ameliorates cognitive deficits in a mouse model of Alzheimer's disease, Nat. Neurosci. 11 (2008) 1311–1318.