0022-3565/09/3311-14–22$20.00
THE JOURNAL OF PHARMACOLOGY AND EXPERIMENTAL THERAPEUTICS
Vol. 331, No. 1
154724/3512655
Printed in U.S.A.

JPET 331:14–22, 2009

The Journal of PHARMACOLOGY And Experimental Therapeutics

Matrix Metalloprotease-9 Inhibition Improves Amyloid β-Mediated Cognitive Impairment and Neurotoxicity in Mice[S]

Hiroyuki Mizoguchi, Kazuhiro Takuma, Emiko Fukuzaki, Daisuke Ibi, Eiichi Someya, Ko-hei Akazawa, Tursun Alkam, Hiroko Tsunekawa, Akihiro Mouri, Yukihiro Noda, Toshitaka Nabeshima, and Kiyofumi Yamada

Laboratory of Neuropsychopharmacology, Division of Life Sciences, Graduate School of Natural Science and Technology, Kanazawa University, Kanazawa, Japan (H.M., K.T., E.F., D.I., E.S., K.A., K.Y.); Futuristic Environmental Simulation Center, Research Institute of Environmental Medicine, Nagoya University, Nagoya, Japan (H.M.); Laboratory of Medicinal Pharmacology, Graduate School of Pharmaceutical Sciences, Osaka University, Osaka, Japan (K.T.); Department of Neuropsychopharmacology and Hospital Pharmacy, Nagoya University Graduate School of Medicine, Nagoya, Japan (D.I., T.A., H.T., A.M., K.Y.); Division of Clinical Science in Clinical Pharmacy Practice, Management and Research, Faculty of Pharmacy, Meijo University, Nagoya, Japan (Y.N.); and Department of Chemical Pharmacology, Graduate School of Pharmaceutical Sciences, Meijo University, Nagoya, Japan (T.N.)

Received April 7, 2009; accepted July 7, 2009

ABSTRACT

In Alzheimer's disease (AD), the expression of matrix metalloproteases (MMPs), which are capable of degrading extracellular matrix proteins, is increased in the brain. Previous studies with cultured glial cells have demonstrated that amyloid β (Aβ) protein can induce the expression of MMPs, which could be involved in the degradation of Aβ. In the present study, we investigated the role of MMP-2 and MMP-9 in cognitive impairment induced by the injection of Aβ in mice. The intracerebroventricular injection of Aβ25-35, Aβ1-40, and Aβ1-42, but not Aβ40-1, transiently increased MMP-9, but not MMP-2, activity and protein expression in the hippocampus. Immunohistochemistry revealed the expression of MMP-9 to be increased in both neurons and glial cells in the hippocampus after Aβ treatment. The Aβ-induced cognitive impairment in vivo as well as neurotoxicity in vitro was significantly alleviated in MMP-9 homozygous knockout mice and by treatment with MMP inhibitors. These results suggest the increase in MMP-9 expression in the hippocampus to be involved in the development of cognitive impairment induced by Aβ1-40. Thus, specific inhibitors of MMP-9 may have therapeutic potential for the treatment of AD. Our findings suggest that, as opposed to expectations based on previous findings, MMP-9 plays a causal role in Aβ-induced cognitive impairment and neurotoxicity.

This study was supported in part by Grants-in-Aid for Scientific Research [Grants 18790052, 19390062]; the 21st Century Center of Excellence Program from the Ministry of Education, Culture, Sports, Science and Technology of Japan; a grant from the Smoking Research Foundation, Japan; the Mochida Memorial Foundation for Medical and Pharmaceutical Research; the Suzuken Memorial Foundation; the Kanzawa Medical Research Foundation; the Japan Society for the Promotion of Science and Korea Science and Engineering Foundation under the Japan-Korea Basic Scientific Cooperation Program; the Academic Frontier Project for Private Universities; matching fund subsidy from Ministry of Education, Culture, Sports, Science and Technology [Grant 2007-2011]; and the Research on Risk of Chemical Substances, Health and Labor Science Research grants supported by the Ministry of Health, Labor and Welfare.

Article, publication date, and citation information can be found at http://jpet.aspetjournals.org.

doi:10.1124/jpet.109.154724.

[S] The online version of this article (available at http://jpet.aspetjournals.org) contains supplemental material.

Alzheimer's disease (AD), the most common neurodegenerative disorder in humans, is characterized by deterioration of cognitive and mental functions, including learning and memory. The formation of extracellular deposits of amyloid β (Aβ) peptide, leading to the formation of neuritic plaques and neurofibrillary tangles in the cortex and hippocampus, is a prominent pathological feature of AD (Yamada and Nabeshima, 2000; Selkoe and Schenk, 2003). Aβ, a spontaneously aggregating peptide of 39 to 43 amino acids, is the primary protein component of senile plaques, a pathological hallmark of AD (Hardy and Selkoe, 2002; Takuma et al., 2005a). Neurotoxicity mediated by Aβ has been well demonstrated both in vivo and in vitro and has been shown to involve oxidative stress, the perturbation of intracellular cal-

ABBREVIATIONS: AD, Alzheimer's disease; Aβ, amyloid β; AAV/Aβ, a viral vector carrying Aβ cDNA; MMP, matrix metalloprotease; LTP, long-term potentiation; MK-801, 5*H*-dibenzo[*a*,*d*]cyclohepten-5,10-imine (dizocilpine maleate); GM6001, *N*-[(2*R*)-2-(hydroxamidocarbonylmethyl)-4-methylpentanoyl]-L-tryptophan methylamide; NORT, novel-object recognition test; FITC, fluorescein isothiocyanate; PBS, phosphate-buffered saline; NeuN, neuron-specific nuclear antigen; GFAP, glial fibrillary acidic protein; LDH, lactate dehydrogenase; ANOVA, analysis of variance; NMDA, *N*-methyl-D-aspartate.

cium homeostasis, and the activation of an apoptotic pathway (Takuma et al., 2005a).

In animal experiments, we have demonstrated that the intrahippocampal injection of Aβ, including Aβ1-40, Aβ1-42, and Aβ25-35, induces hippocampal damage, learning, and memory deficits (Yamada et al., 2005; Alkam et al., 2007; Wang et al., 2007), and impairment of the cholinergic system, which play important roles in cognitive deficits associated with aging and neurodegenerative diseases (Yamada et al., 1999; Tran et al., 2001). A recent study has shown that intraventricular infusion of Aβ1-42 induces learning deficits in 9-month-old but not 2.5-month-old mice, and these learning deficits are shown 12, but not 6, weeks after infusion of Aβ1-42 in 9-month-old mice, suggesting that Aβ infusion results in age-dependent and delayed learning deficits without role of Aβ deposition and inflammation (Malm et al., 2006). In addition, we have shown that the oral administration of a viral vector carrying Aβ cDNA (AAV/Aβ) reduced the amount of Aβ accumulated and attenuated cognitive impairment in Tg2576 mice, suggesting AAV/Aβ to be safe and effective for the treatment of AD and that the accumulation of Aβ is the event initiating the decades-long pathological cascade leading to the disease (Mouri et al., 2007). Whereas plaques and amyloid fibrils have been viewed by some as resistant to proteolytic degradation, it is possible that certain proteases contribute to endogenous mechanisms leading to the clearance of plaques.

Matrix metalloproteinases (MMPs) function to remodel the pericellular environment, primarily through the cleavage of extracellular matrix proteins and cell surface components (Yong et al., 2001). Gelatinases (MMP-2 and MMP-9) are capable of cleaving collagen IV and V, laminin, and chondroitin sulfate proteoglycan, which are associated with cell adhesion (Yong et al., 2001). Furthermore, MMP-9 degrades Aβ and amyloid plaques (Yan et al., 2006) and has been implicated specifically in cerebral ischemia (Lo et al., 2002), kainate-induced neuronal injury (Szklarczyk et al., 2002), hippocampal long-term potentiation (LTP) and memory (Nagy et al., 2006), and methamphetamine dependence (Mizoguchi et al., 2007a,b). Thus, gelatinases are involved in neuronal activity-dependent synaptic plasticity and cell death in the brain.

It is interesting that MMP-9 is increased in the brains of AD patients (Backstrom et al., 1996). Moreover, MMP-9 expression in astrocytes is induced in the presence of Aβ peptide (Deb et al., 2003). MMP-9 is expressed in the cytoplasm of neurons, neurofibrillary tangles, senile plaques, and vascular walls in brain tissue from AD patients (Asahina et al., 2001). Although MMP-9 has been found to cleave the Aβ peptide at several sites (Backstrom et al., 1996; Yan et al., 2006), its potential role in Aβ-induced cognitive dysfunction and neurotoxicity has not yet been elucidated.

In the present study, we investigated the role of MMP-2 and MMP-9 in cognitive impairment induced by the intracerebroventricular injection of Aβ in mice, as well as Aβ-induced neurotoxicity in primary cultured neurons. Our findings suggest that, as opposed to expectations based on previous findings, MMP-9 plays a causal role in Aβ-induced cognitive impairment and neurotoxicity.

Materials and Methods

Animals. Male ICR mice (6 weeks old; Charles River Japan, Yokohama, Japan), weighing 20 ± 5 g at the beginning of the experiments, were used. We also used MMP-9 homozygous knockout [MMP-9(−/−)] mice and wild-type (FVB/N) mice (10–12 weeks old) obtained from The Jackson Laboratory (Bar Harbor, ME).

All experiments were performed in accordance with the Guidelines for Animal Experiments of the Kanazawa University (Kanazawa, Japan) and Nagoya University Graduate School of Medicine (Nagoya, Japan), the Guiding Principles for the Care and Use of Laboratory Animals approved by the Japanese Pharmacological Society (Tokyo, Japan), and the National Institutes of Health *Guide for the Care and Use of Laboratory Animals* (National Institutes of Health, Bethesda, MD).

Drugs. The doses of all drugs are expressed as those of the salt. Aβ25-35, Aβ1-40, and Aβ40-1 (Bachem California, Torrance, CA) were dissolved in distilled water at a concentration of 1 mg/ml and stored at −30°C before use and incubated for aggregation at 37°C for 4 days before the injection. Aβ peptides were injected intracerebroventricular at a volume of 3 μl. Vehicle and Aβ40-1 were injected as the control. All peptides were injected as described previously (Alkam et al., 2007; Wang et al., 2007). In brief, a microsyringe with a 28-gauge stainless steel needle 3.0 mm long was used for all experiments. Mice were anesthetized lightly with ether, and the needle was inserted unilaterally 1 mm to the right of the midline point equidistant from each eye, at an equal distance between the eyes and the ears and perpendicular to the plane of the skull. A single shot of the same volume (3 μl) of peptide or vehicle was delivered gradually within 3 min. Mice exhibited normal behavior within 1 min after the injection. MK-801 (Sigma-Aldrich, St. Louis, MO) at a dose of 0.1 to 0.3 mg/kg was given 30 min before Aβ25-35. GM6001 (Calbiochem, San Diego, CA) at a dose of 5 μg was intracerebroventricularly injected with Aβ in a total volume of 5 μl.

Novel-Object Recognition Test. The NORT was carried out as described previously (Mizoguchi et al., 2008). The experimental apparatus consisted of a Plexiglas open-field box (30 × 30 × 35 cm high), with a sawdust-covered floor. The apparatus was located in a sound-attenuated room and was illuminated with a 20-W bulb.

In a standard procedure, the NORT consisted of three sessions: habituation, training, and retention. Each mouse was individually habituated to the box, with 10 min of exploration in the absence of objects for two consecutive days (habituation session, days 1–2). During the training session, two novel objects were symmetrically fixed to the floor of the box, 8 cm from the walls, and each animal was allowed to explore in the box for 10 min (day 3). The objects were constructed from a golf ball, wooden column, and wall socket, which were different in shape and color but similar in size. An animal was considered to be exploring the object when its head was facing the object or it was touching or sniffing the object. The time spent exploring each object was recorded. After training, mice were immediately returned to their home cages. During the retention sessions, the animals were placed back into the same box 24 h after the training session (day 4), but one of the familiar objects used during training had been replaced with a novel object. The animals were then allowed to explore freely for 5 min, and the time spent exploring each object was recorded. Throughout the experiments, the objects were used in a counterbalanced manner in terms of their physical complexity and emotional neutrality. A preference index in the retention session, a ratio of the amount of time spent exploring the novel object over the total time spent exploring both objects, was used to measure cognitive function. In the training session, the preference index was calculated as a ratio of the time spent exploring the object that was replaced by the novel object in the retention session, over the total exploring time.

Repeated Training NORT. The procedure of repeated training NORT is the same with the standard NORT, except that the number of training sessions was increased from one to four, and then the

mice were subjected to the retention session. During the four training sessions (days 3–4, twice a day), mice were repeatedly exposed to the same two objects in the test box. During the retention session (day 5), one of the two familiar objects used during four training sessions was replaced with a novel object.

Gel Zymography. Samples were prepared as described previously (Mizoguchi et al., 2007a,b). In brief, brain tissues were homogenized in lysis buffer (50 mM Tris-HCl, 150 mM NaCl, 5 mM $CaCl_2$, 0.05% Brij 35, and 0.02% NaN_3, pH 7.6) with 1% Triton X-100 and centrifuged at 12,000*g* for 10 min to pellet insoluble material. The protein concentration in the supernatant was determined using a Protein Assay Rapid kit (Bio-Rad, Osaka, Japan). The supernatant was incubated with gelatin-Sepharose 4B (GE Healthcare, Chalfont St. Giles, Buckinghamshire, UK) that had been washed three times with the lysis buffer, with constant shaking, for 24 h at 4°C. After centrifugation at 500*g* for 2 min, the pellet was resuspended in 500 μl of the lysis buffer and washed three times. The pellet was resuspended in 150 μl of lysis buffer containing 10% dimethyl sulfoxide and shaken for 2 h, and then it was used for assaying gelatinase activity of MMP-2 and MMP-9.

The samples were subjected to electrophoresis in a 10% SDS-polyacrylamide gel electrophoresis containing 0.1% gelatin under nonreducing conditions. Gels were washed twice for 30 min in 2.5% Triton X-100 to remove SDS, washed for 30 min in incubation buffer (50 mM Tris-HCl, 5 mM $CaCl_2$, 2 μM $ZnCl_2$, 200 mM NaCl, and 0.02% Briji 35, pH 7.4) at room temperature, and further incubated for 24 h in the same buffer at 37°C. Gels were then stained for 3 h in Coomassie Blue (1% Coomassie Brilliant Blue G-250, 30% methanol, and 10% acetic acid) and destained in 40% methanol/7% acetic acid until clear bands of gelatinolysis occurred on a dark background. Total activity including pro-MMP activity was analyzed with the ATTO Densitograph Software Library Lane Analyzer (Atto Instruments, Tokyo, Japan).

Western Blotting. Brain tissues were homogenized in lysis buffer (20 mM Tris-HCl, 100 mM NaCl, 1 mM $CaCl_2$, and 0.005% Brij 35, pH 7.4) and microwaved for 15 s according to the protocol for immunoblotting with monoclonal antibodies. The homogenate was centrifuged at 13,000*g* for 30 min to pellet insoluble material. The protein concentration in the supernatant was determined using a Protein Assay Rapid kit. The sample was boiled in 2× sample buffer (0.25% bromphenol blue, 12% 2-mercaptothanol, 20% glycerol, 4% SDS, and 0.1 M Tris-HCl, pH 6.8) and subjected to SDS-polyacrylamide gel electrophoresis on a 4% stacking gel and 8% separating gel, and then transferred electrophoretically to a polyvinylidene difluoride membrane (Millipore, Billerica, MA). The same concentration (20–40 μg) of protein per lane was loaded for all Western blotting. The band intensities of the film were analyzed by densitometry. The amount of MMP-9 was calculated versus the amount of β-actin protein. The primary polyclonal rabbit or goat antibodies used in the present study were anti-MMP-9 (1:1000; Abcam plc, Cambridge, UK) and β-actin (1:2000; Santa Cruz Biotechnology, Inc., Santa Cruz, CA). The secondary antibodies, horseradish peroxidase-linked anti-rabbit and anti-goat IgG, were used at 1:2000 and 1:5000 dilutions, respectively (Kirkegaard and Perry Laboratories, Gaithersburg, MD). Immunoreactive materials on the membrane were detected using enhanced chemiluminescence Western blotting detection reagents (GE Healthcare) and exposed to X-ray film. The band intensities of the film were analyzed by densitometry.

In Situ Zymography. Mice were intracardially perfused with ice-cold saline before being frozen at −80°C using optimal cutting temperature compound (Sakura Finetechnical, Tokyo, Japan). The brains were sectioned at 20 μm in a cryostat. We adapted an in situ zymography method to localize net gelatinolytic activity in brain sections as described previously (Szklarczyk et al., 2002; Mizoguchi et al., 2007b). Nonfixed sections were incubated for 24 h at 37°C in a humid dark chamber with a reaction buffer containing 0.5 M Tris-HCl, 1.5 M NaCl, 50 mM $CaCl_2$, and 2 mM sodium azide, pH 7.6, and 100 μg/ml FITC-labeled DQ-gelatin (Invitrogen, Carlsbad, CA) intramolecularly quenched. After the incubation, sections were washed in PBS, fixed in 4% paraformaldehyde, and mounted on slides. Some sections were incubated with the broad-spectrum MMP inhibitor 1,10-*O*-phenanthroline (1 mM; Invitrogen). Samples were observed with an FITC filter, and the images were analyzed using an AxioVision 3.0 system (Carl Zeiss, Jena, Germany). The cleavage of gelatin-FITCs by tissue gelatinases releases quenched fluorescence representative of net proteolytic activity. Sections incubated without FITC-labeled DQ-gelatin were not fluorescent.

Double Immunostaining. Polyclonal rabbit anti-MMP-9 antibody (1:250; Abcam plc), monoclonal mouse anti-neuron-specific nuclear antigen (NeuN) antibody (1:200; Millipore Bioscience Research Reagents, Temecula, CA), anti-glial fibrillary acidic protein (GFAP) antibody (1:200; Millipore Bioscience Research Reagents), and anti-F8/40 antibody (1:100; Sigma-Aldrich) served as primary antibodies. Affinity-purified FITC-conjugated goat anti-rabbit IgG and rhodamine-conjugated goat anti-mouse IgG served as secondary antibodies. Samples were observed with an AxioVision 3.0 system.

Neuronal Cultures. Cortical neurons were prepared from mouse embryos at 17 days of gestation as described previously (Takuma et al., 2005b). In brief, slices of cerebral cortex were digested with trypsin and triturated in Neurobasal medium containing 2% B27 supplement, 0.5 mM glutamine, and 4.4 mM sodium bicarbonate at 4°C. Cells were separated from debris and diluted in 1 ml of the medium. After centrifugation for 2 min, the cell pellet was resuspended in Neurobasal medium containing 2% B27 supplement, 0.5 mM glutamine, 50 U/ml penicillin, and 50 μg/ml streptomycin, and plated at 2×10^6 cells/ml into 24-well plates coated with 50 μg/ml poly(D-lysine).

Aβ1-40 (Peptide Institute Inc., Osaka, Japan) at a concentration of 10 μM was added to cultured cortical neurons from ICR mice for 24 h. Cells were fixed with 3% paraformaldehyde in PBS(−) at 4°C and washed three times. Polyclonal rabbit anti-MMP-9 antibody and monoclonal mouse anti-NeuN antibody served as primary antibodies in 1% bovine serum albumin and 0.1% Triton X-100 in PBS(−). Affinity-purified FITC-conjugated goat anti-rabbit IgG and rhodamine-conjugated goat anti-mouse IgG served as secondary antibodies. Samples were observed with an AxioVision 3.0 system and LSM 510 confocal microscope (Carl Zeiss).

Cell Death Assay. Cell death was evaluated by measuring lactate dehydrogenase (LDH) activity released into the medium using a colorimetric assay as described previously (Takuma et al., 2005b). In brief, cells in 96-well plates were exposed to experimental treatments, and then 50 μl of culture supernatant was collected from each well. Supernatants were reacted with a tetrazolium salt at room temperature for 30 min, and stop solution was added. Absorbance at 405 nm was measured on a Benchmark microplate reader (Bio-Rad). Total cellular LDH activity was determined by lysing the cells. The amount of LDH activity released from cells was expressed as a percentage of total LDH activity.

An MMP-2/-9 inhibitor II (Calbiochem) at doses of 10 and 30 μM was added to the culture medium 30 min before treatment with Aβ1-40 at a dose of 10 μM in cultured cortical neurons from ICR mice. Three or 5 days after treatment, the amount of LDH released from the cultured neurons was measured. Total cellular LDH activity was determined by lysing the cells. The amount of LDH activity released from the cells was expressed as a percentage of total LDH activity.

Statistical Analysis. All data are expressed as the mean ± S.E. Statistical significance was determined using a one-way analysis of variance (ANOVA), followed by the Student-Newman-Keuls test for multigroup comparisons. p values less than 0.05 were taken to indicate statistically significant differences. Student's t test was used for two-group comparisons.

Results

Dose- and Time-Dependent Changes in MMP-9 Activity in the Hippocampus after Intracerebroventricular Injection of Aβ25-35 or Aβ1-40 in Mice. We investigated whether MMP-2 and MMP-9 activities were induced by the intracerebroventricular injection of Aβ25-35 and Aβ1-40 using gel zymography method. In Fig. 1A, lane 8 represents a zymographic control marker, murine recombinant MMP-9, whose molecular mass is approximately 105 kDa. The injection of Aβ25-35 transiently and dose-dependently increased MMP-9, but not MMP-2, activity in the hippocampus compared with the activity in the vehicle-treated control group [$F_{(2,22)} = 0.84, p > 0.05$ (Fig. 1B); $F_{(2,22)} = 7.58, p < 0.05$ (Fig. 1C) by one-way ANOVA]. MMP-9 activity was markedly increased to 500 to 1300% of control levels on day 1, but it returned to the basal levels on days 3 and 7 after injection. Likewise, the intracerebroventricular injection of Aβ1-40 dose-dependently increased MMP-9, but not MMP-2, activity in the hippocampus [$F_{(4,33)} = 0.25$, $p > 0.05$ (Fig. 2B); $F_{(4,33)} = 3.22, p < 0.05$ (Fig. 2C) by one-way ANOVA] on day 1. Again, the MMP-9 activity returned to the basal levels of vehicle or Aβ40-1-injected mice on days 3 and 7 ($p < 0.05$ by t test; Fig. 2F). It should be noted that the injection of Aβ25-35 and Aβ1-40 produced a lower molecular weight band that may be an active form of MMP-9 (Figs. 1A and 2, A and D). Moreover, the intracerebroventricular injection of Aβ1-40 tended to increase MMP-9, but not MMP-2, activity in the frontal cortex on day 1, but the change was not statistically significant (Supplemental Fig. 1). It was confirmed that the intracerebroventricular injection of the more fibrinogenic Aβ1-42 at 900 pmol significantly increased MMP-9 activity in the hippocampus on day 1 after injection [vehicle-injected control mice ($n = 6$), 100 ± 27; Aβ1-42-injected mice ($n = 8$), 460 ± 57]. The injection of Aβ1-42 also induced a minimal increase in MMP-2 activity [vehicle-injected control mice ($n = 6$), 100 ± 5; Aβ1-42-injected mice ($n = 8$), 120 ± 4].

Spatial Changes in Net Proteolytic Activity in the Hippocampus after Intracerebroventricular Injection of Aβ1-40. We analyzed the spatial changes in gelatinase

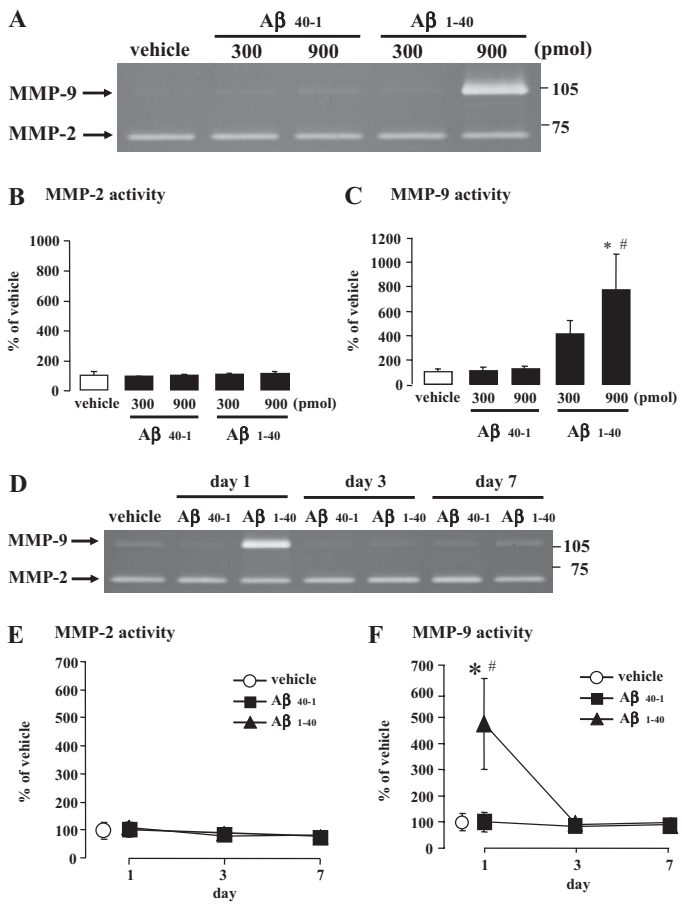

Fig. 2. Effect of intracerebroventricular injection of Aβ1-40 on MMP-2 (B and E) and MMP-9 (C and F) activities in the hippocampus. A and D, gel zymography. Mice were injected with either vehicle or Aβ1-40 at a dose of 300 or 900 pmol and then killed on day 1, 3, and 7 after the injection. Values are the mean ± S.E. [B and C; vehicle, $n = 8$; Aβ40-1 (300 pmol), $n = 4$; Aβ40-1 (900 pmol), $n = 8$; Aβ1-40 (300 pmol), $n = 9$; Aβ1-40 (900 pmol), $n = 9$; E and F, vehicle, $n = 11$; Aβ40-1 (900 pmol; day 1), $n = 12$; Aβ40-1 (900 pmol; day 3) $n = 4$; Aβ40-1 (900 pmol; day 7), $n = 4$; Aβ1-40 (900 pmol; day 1), $n = 13$; Aβ1-40 (900 pmol; day 3), $n = 4$; Aβ1-40 (900 pmol; day 7), $n = 4$]. *, $p < 0.05$ versus vehicle-injected mice. #, $p < 0.05$ versus Aβ40-1-injected mice (day 1).

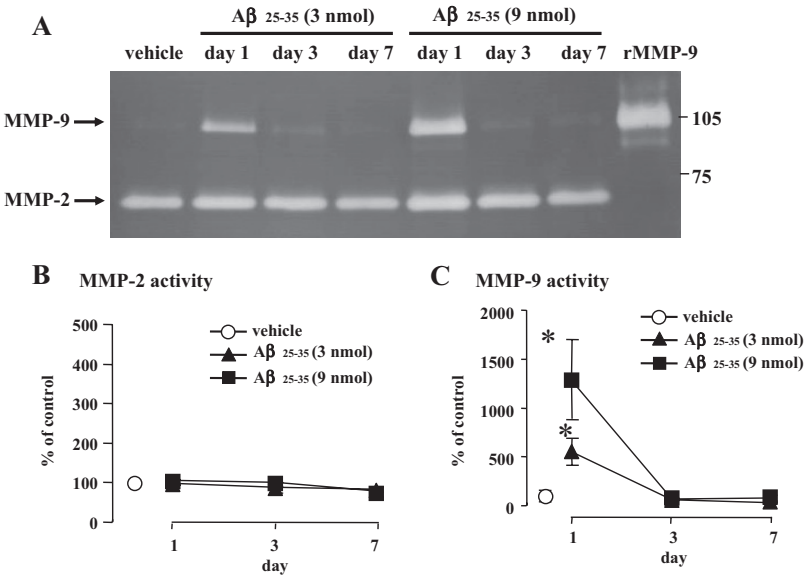

Fig. 1. Effect of intracerebroventricular injection of Aβ25-35 on MMP-2 (B) and MMP-9 (C) activities in the hippocampus. A, gel zymography. Mice were injected with either vehicle or Aβ25-35 at a dose of 3 or 9 nmol and then killed on day 1, 3, and 7 after the injection. Values are the mean ± S.E [vehicle; $n = 9$; Aβ25-35 (3 nmol; day 1), $n = 8$; Aβ25-35 (3 nmol; day 3), $n = 5$; Aβ25-35 (3 nmol; day 7), $n = 3$; Aβ25-35 (9 nmol; day 1), $n = 8$; Aβ25-35 (9 nmol; day 3), $n = 4$; Aβ25-35 (9 nmol; day 7), $n = 3$]. *, $p < 0.05$ versus vehicle-injected mice.

activity in the hippocampus after the intracerebroventricular injection of Aβ1-40 by in situ zymography. Brain sections were incubated with gelatin conjugated to a quenched fluorescence dye, and the cleavage of gelatin by gelatinase results in an increase in fluorescence. The signal was completely inhibited by the zinc chelator phenantroline, a broad-spectrum MMP inhibitor, indicating that the fluorescence is associated with MMP activity (Supplemental Fig. 2, compare B with A). In vehicle- and Aβ40-1-injected groups, gelatinase activity was observed in the CA1–CA4 layers and dentate gyrus of the hippocampus, indicating that constitutive gelatinolytic activity was localized to the main neuronal layers of the hippocampus. Twenty-four hours after the intracerebroventricular injection of Aβ1-40, an intense signal was visualized in the hippocampus compared with the Aβ40-1-injected group (Supplemental Fig. 2, compare D with C). In addition, the gelatinase activity was markedly increased in the molecular layer of the hippocampus in the Aβ1-40-treated group compared with the Aβ40-1-injected group (Supplemental Fig. 2, E and F).

The Intracerebroventricular Injection of Aβ1-40 Increases MMP-9 Protein Expression in the Brain. Next, we examined whether MMP-9 protein levels were also increased in the hippocampus by the intracerebroventricular injection of Aβ1-40 on day 1. A Western blot analysis revealed the hippocampal protein level to be increased 24 h after Aβ1-40 was injected at 900 pmol compared with levels in the vehicle- and Aβ40-1-treated groups [$F_{(2,15)} = 5.55$, $p < 0.05$ (Fig. 3A) by one-way ANOVA].

To determine the cell types in which the expression of MMP-9 is induced by the injection of Aβ1-40, double immunostaining for MMP-9 with NeuN, a neuronal marker (Fig. 3D); F4/80, a microglial marker (Fig. 3E); or GFAP, an astroglial marker (Fig. 3F), was performed. In the Aβ1-40-treated group, strong immunolabeling of MMP-9 was visualized in the CA3 layers of the hippocampus compared with the Aβ40-1-injected group (Fig. 3, compare B with C), in which the majority of the immunoreactivity was colocalized to NeuN-positive cells, suggesting the expression of MMP-9 in neurons. However, some MMP-9 immunoreactivity was observed in F4/80- or GFAP-positive cells in the hippocampus (Fig. 3, D–F).

Role of the Aβ-Induced Increase in MMP-9 Expression in Aβ-Induced Impairment of Recognition Memory. To examine the role of MMP-9 in Aβ1-40-induced cognitive dysfunction, we investigated the effect of GM6001, a broad-spectrum MMP inhibitor (Galardy et al., 1994; Wang and Tsirka, 2005), on Aβ1-40-induced impairment of recognition memory in the NORT. Cotreatment with GM6001 dose-dependently suppressed the Aβ1-40-induced increase in hippocampal MMP-9 activity compared with 2.5% dimethyl sulfoxide, and the effect of GM6001 (5 μg) was statistically significant [$F_{(2,16)} = 3.80$, $p < 0.05$ (Supplemental Fig. 3A) by one-way ANOVA]. However, GM6001 had little effect on MMP-2 activity, and there was no significant difference in activity between the 2.5% dimethyl sulfoxide-injected and GM6001-injected mice (Supplemental Fig. 3A). As shown in Supplemental Fig. 3B, the intracerebroventricular injection of Aβ1-40 significantly reduced exploratory preference for the novel object in the retention session [$F_{(3,18)} = 5.68$, $p < 0.05$ (Supplemental Fig. 3B) by one-way ANOVA], without affecting total exploration time in the training and retention sessions [data not shown; training: $F_{(3,18)} = 0.29$, $p > 0.05$ by one-way ANOVA; retention: $F_{(3,18)} = 2.05$, $p > 0.05$ by one-way ANOVA], indicating the impairment of recognition memory in Aβ1-40-injected mice. Simultaneous treatment with GM6001 (5 μg) in Aβ1-40-injected mice caused a significant improvement in exploratory preference in the retention session [$F_{(3,18)} = 5.68$, $p < 0.05$ (Supplemental Fig. 3B) by one-way ANOVA], without affecting the exploratory preference in the training session [$F_{(3,18)} = 0.34$, $p > 0.05$ (Supplemental Fig. 3B) by one-way ANOVA] or total exploration time in the training or retention session [data not shown; training: $F_{(3,18)} = 0.29$, $p > 0.05$ by one-way ANOVA; retention: $F_{(3,18)} = 2.05$, $p > 0.05$ by one-way ANOVA].

In addition to the pharmacological studies, we investigated the role of MMP-9 in Aβ1-40-induced impairment of recognition memory using MMP-9(−/−) mice. In the standard (one-training) procedure of NORT, the exploratory preference to the novel object in the retention session was markedly reduced in MMP-9(−/−) mice (49.3 ± 2.9; $n = 10$) compared with wild-type mice (69.0 ± 2.3; $n = 10$) without a change in total exploration time in the training and retention sessions. Thus, it was difficult to assess the Aβ-induced memory impairment in MMP-9(−/−) mice in a standard one-training NORT. Accordingly, the number of training sessions was increased from one to four, and the mice were subjected to the retention session (repeated training NORT). There was no difference in total exploration time in the first and fourth training sessions between Aβ40-1-treated wild-type and MMP-9(−/−) mice, respectively [first training: $F_{(3,21)} = 2.28$, $p > 0.05$ by one-way ANOVA; fourth training: $F_{(3,21)} = 1.75$, $p > 0.05$ by one-way ANOVA] (Fig. 4A). As shown in Fig. 4B, there was no difference in exploratory preference in the retention session between Aβ40-1-treated wild-type and MMP-9(−/−) mice, indicating that the Aβ40-1-treated MMP-9(−/−) mice could recognize the novel object 24 h after four training sessions [$F_{(3,21)} = 17.9$, $p < 0.05$ by one-way ANOVA for retention] (Fig. 4B). Under these conditions, Aβ1-40-injected wild-type mice showed a marked impairment of exploratory preference in the retention session [$F_{(3,21)} = 17.9$, $p < 0.05$ by one-way ANOVA] (Fig. 4B), without exhibiting a change in exploratory preference in the training sessions [first training: $F_{(3,21)} = 2.00$, $p > 0.05$ by one-way ANOVA; fourth training: $F_{(3,21)} = 1.99$, $p > 0.05$ by one-way ANOVA] (Fig. 4B) or in total exploration time in the training sessions [first training: $F_{(3,21)} = 2.28$, $p > 0.05$ by one-way ANOVA;

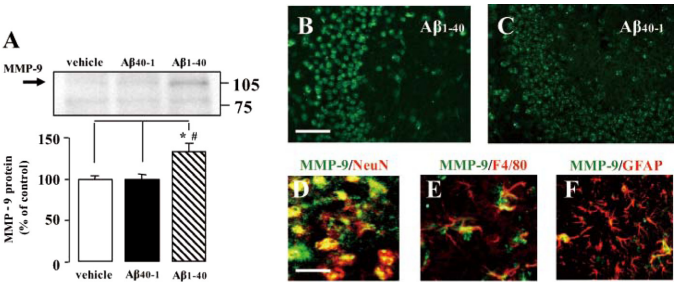

Fig. 3. Effect of intracerebroventricular injection of Aβ1-40 on the MMP-9 protein level in the hippocampus. A, Western blot analysis: Mice were injected with either vehicle, Aβ40-1, or Aβ1-40 at a dose of 900 pmol and then killed 1 day later. Values are the mean ± S.E. (A; $n = 6$). *, $p < 0.05$ versus vehicle-injected mice. #, $p < 0.05$ versus Aβ40-1-injected mice. B to F, double immunostaining for MMP-9 (B–F; green) and NeuN (D; red), F4/80 (E; red), or GFAP (F; red) in the CA3 layer of the hippocampus. Scale bar, 100 μm (B) and 10 μm (D).

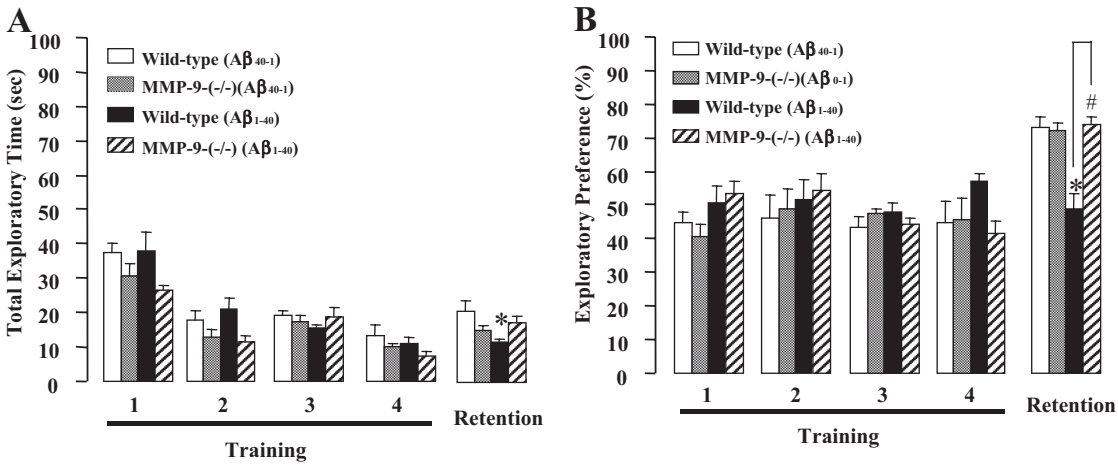

Fig. 4. Effect of Aβ1-40 on total exploratory time (A) and exploratory preference (B) in repeated training NORT in wild-type and MMP-9(−/−) mice. Mice were intracerebroventricularly injected with Aβ1-40 at a dose of 900 pmol. Values are the mean ± S.E. (n = 5–7). *, $p < 0.05$ versus wild type (Aβ40-1). #, $p < 0.05$ versus wild type (Aβ1-40).

fourth training: $F_{(3,21)} = 1.75$, $p > 0.05$ by one-way ANOVA] (Fig. 4A). There was a slight but significant difference in total exploration time in the retention session between Aβ40-1- and Aβ1-40-injected wild-type mice [$F_{(3,21)} = 4.86$, $p < 0.05$ by one-way ANOVA] (Fig. 6A).

Pretreatment with MK-801 Inhibits Aβ25-35-Induced Increase in MMP-9 Expression and Impairment of Recognition Memory. To examine the mechanism by which Aβ injection induces the MMP expression, we investigated the effect of MK-801, a noncompetitive *N*-methyl-D-aspartate (NMDA) receptor antagonist, on the Aβ25-35-induced increase in MMP-9 expression and impairment of recognition memory in the NORT. Pretreatment with MK-801 had no effect on the hippocampal MMP-9 activity in vehicle-treated mice. However, the NMDA receptor antagonist completely inhibited the Aβ25-35-induced increase in MMP-9 expression in the hippocampus [$F_{(3,15)} = 6.17$, $p < 0.05$ by one-way ANOVA] (Fig. 5A). Moreover, pretreatment with MK-801 (0.1–0.3 mg/kg) in Aβ25-35-injected mice caused a dose-dependent and significant improvement of exploratory preference in the retention session [$F_{(5,51)} = 2.74$, $p < 0.05$ by one-way ANOVA] (Fig. 5B), without affecting exploratory preference in the training session [$F_{(5,51)} = 0.65$, $p > 0.05$ by one-way ANOVA] (Fig. 5B) or total exploration time in the training [$F_{(5,51)} = 0.93$, $p > 0.05$ by one-way ANOVA] or retention session [$F_{(5,51)} = 0.87$, $p > 0.05$ by one-way ANOVA]. Post hoc analysis indicated that MK-801 at 0.3 mg/kg, but not 0.1 mg/kg, significantly improved exploratory preference in Aβ25-35-treated mice ($p < 0.05$). MK-801 at 0.1 to 0.3 mg/kg in the vehicle-treated control group had no effect on exploratory preference or total exploration time in the training and retention sessions (Fig. 5B).

Effect of Aβ1-40 on MMP-9 Expression in Primary Cultured Cortical Neurons. Treatment with Aβ1-40 (Supplemental Fig. 4E), but not Aβ40-1 (Supplemental Fig. 4C), at a dose of 10 μM for 24 h induced MMP-9 expression in primary cultured cortical neurons compared with vehicle treatment (Supplemental Fig. 4A). Immunoreactivity was observed in NeuN-positive cells, indicating that Aβ1-40 treatment can induce MMP-9 expression in neurons (Supplemental Fig. 4G). NeuN-positive cells differed between the Aβ1-40- (Supplemental Fig. 4F) and Aβ40-1-treated (Supplemental Fig. 4D) groups, suggesting that cell death was not induced drastically 24 h after Aβ1-40 treatment (Supplemental Fig. 4, compare F with D).

A Specific Inhibitor of MMP-2/-9 Attenuated Aβ1-40-Induced Neurotoxicity in Primary Cultured Cortical Neurons. Finally, we investigated the role of MMP-9 in Aβ1-40-induced neurotoxicity by measuring LDH activity released into the culture medium. Aβ1-40 treatment for 3 or 5 days markedly induced the release of LDH from cultured cortical neurons [3 days (Fig. 6A): $F_{(5,18)} = 17.6$, $p < 0.05$ by one-way ANOVA; 5 days (Fig. 6B): $F_{(5,18)} = 40.4$, $p < 0.05$ by one-way ANOVA]. However, cotreatment with rather specific MMP-2/-9 inhibitor reduced the amount of LDH released from cultured cortical neurons [3 days (Fig. 6A): $F_{(5,18)} = 17.6$, $p < 0.05$ by one-way ANOVA; 5 days (Fig. 6B): $F_{(5,18)} =$

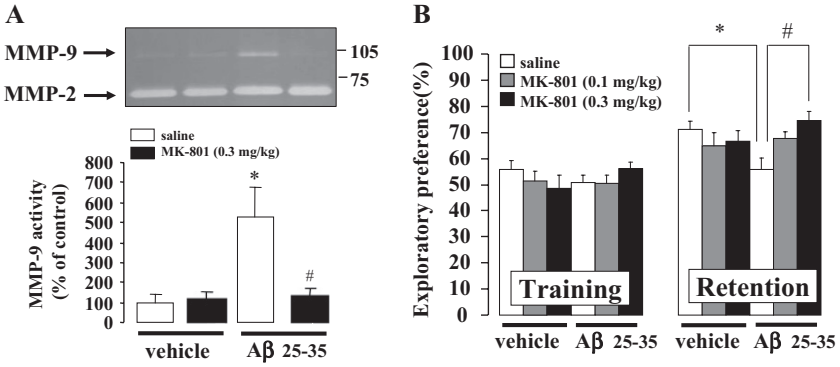

Fig. 5. Effect of MK-801 on Aβ25-35-induced increase in hippocampal MMP-9 activity (A) and memory impairment (B). Mice were given MK-801 at 0.1 to 0.3 mg/kg 30 min before receiving an intracerebroventricular injection of Aβ25-35 at 3 nmol. Values are the mean ± S.E. (n = 4–5 for A; n = 9–10 for B). *, $p < 0.05$ versus saline + vehicle. #, $p < 0.05$ versus saline + Aβ25-35.

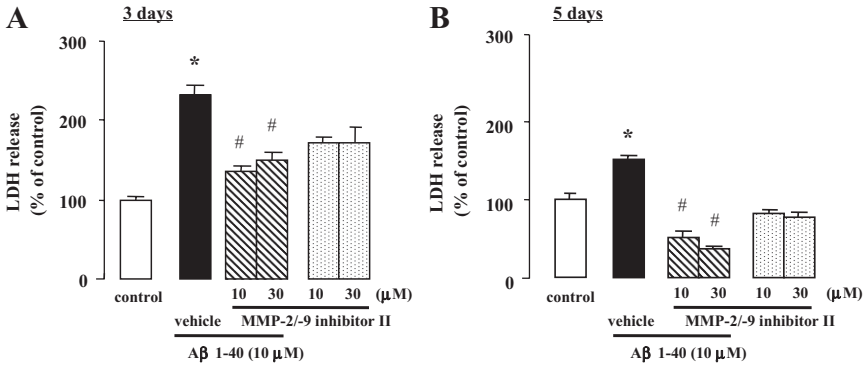

Fig. 6. Effect of a specific MMP-2/-9 inhibitor on Aβ-induced neurotoxicity in primary cultured cortical neurons. A, MMP-2/-9 inhibitor II at a dose of 10 or 30 μM was added simultaneously with Aβ1-40 at a dose of 10 μM to primary cultured cortical neurons from ICR mice. Three or 5 days later, the amount of LDH released from cultured neurons was measured. Total cellular LDH activity was determined by lysing the cells. The amount of LDH activity released from the cells was expressed as a percentage of total LDH activity. Values are the mean ± S.E. for four determinations per experimental condition. *, $p < 0.05$ versus control. #, $p < 0.05$ versus Aβ1-40 + vehicle.

40.4 $p < 0.05$ by one-way ANOVA], although the inhibitor itself had no effect on the release.

In addition to the pharmacological studies, we investigated the role of MMP-9 in Aβ1-40-induced neurotoxicity in primary cultured neurons from MMP-9(−/−) mice. Treatment with Aβ1-40 at 10 μM for 5 days markedly increased the amount of LDH released from cultured cortical neurons of wild-type mice [$F_{(5,20)} = 3.47$, $p < 0.05$ by one-way ANOVA] (Fig. 7). The Aβ1-40-induced release was almost completely suppressed in the neurons from MMP-9(−/−) mice compared with those from wild-type mice (Fig. 7).

Discussion

In the Aβ25-35-, Aβ1-40-, and Aβ1-42-injected mice, MMP-9 activity and protein expression were transiently increased in the hippocampus. We demonstrated using inhibitors of MMPs and MMP-9(−/−) mice that the increase in MMP-9 expression in the hippocampus is associated with the development of cognitive impairment and neurotoxicity induced by Aβ. Thus, specific inhibitors of MMP-9 may have a therapeutic potential for the treatment of AD.

Members of the MMP subfamily, the gelatinases MMP-2 and MMP-9, are initially expressed as inactive proenzymes and cleaved into active forms after cellular release (Van den Steen et al., 2002); this property places these proteases in a unique position to regulate levels of substrates in the extracellular space. Our present study apparently indicated that the activity of MMP-2 was constantly expressed, whereas that of MMP-9 was very weak in the hippocampus of vehicle-injected mice. The intracerebroventricular injection of Aβ led to a transient induction of MMP-9 expression in the hippocampus. In addition, we demonstrated that the Aβ-induced expression of MMP-9 was localized to neuronal and glial cells in the hippocampus. Previous study has revealed that both MMP-2 and MMP-9 are expressed in the presence of Aβ (Deb and Gottschall, 1996) and highly expressed and secreted by astrocytes (Muir et al., 2002; Deb et al., 2003). In contrast, MMP-9 is synthesized in neurons of the human hippocampus (Backstrom et al., 1996) and is expressed in the cytoplasm of neurons, neurofibrillary tangles, vascular walls, and senile plaques in the brain tissues of AD patients (Asahina et al., 2001). Our findings are consistent with these previous reports.

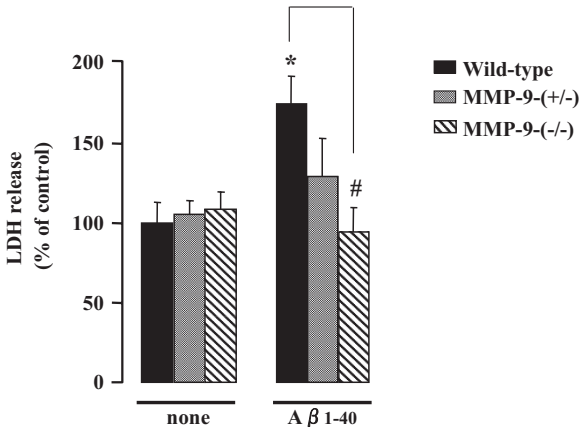

Fig. 7. Aβ-induced neurotoxicity in primary cultured cortical neurons from MMP(−/−) mice. Primary cultured cortical neurons from wild-type, MMP-9(+/−), or MMP-9(−/−) mice were treated with Aβ (10 μM). Five days later, the amount of LDH released from cultured neurons was measured. Total cellular LDH activity was determined by lysing the cells. The amount of LDH activity released from the cells was expressed as a percentage of total LDH activity. Values are the mean ± S.E. for three to five determinations per experimental condition. *, $p < 0.05$ versus control. #, $p < 0.05$ versus Aβ1-40 + vehicle.

To clarify the mechanism underlying Aβ-induced expression of MMP-9, we focused on the role of NMDA receptors, because it has been reported that Aβ activates NMDA receptors (Snyder et al., 2005), and MMP-9 expression is induced via NMDA receptors (Meighan et al., 2006; Nagy et al., 2006; Tian et al., 2007). Pretreatment with MK-801 inhibited the Aβ-induced increase in MMP-9 expression and cognitive impairment, indicating that MMP-9's induction and cognitive dysfunction are induced by Aβ treatment associated at least in part with the activation of NMDA receptors in this model. In fact, an excessive amount of glutamate in the synaptic microenvironment and the persistent influx of Ca^{2+} through NMDA receptors are considered major causes of neurodegeneration in AD (Wenk, 2006). For example, in the rat magnocellular nucleus basalis, Aβ-induced toxicity was effectively reduced by MK-801, and Aβ promoted an excitotoxic pathway that includes astroglial depolarization, extracellular glutamate accumulation, NMDA receptor activation culminating in intracellular Ca^{2+} overload, and cell death (Harkany et al., 2000). Direct injection of Aβ1-40 in the hippocampus caused neuronal loss in the CA1 area and treatment with an NMDA antagonist, memantine, reduced the Aβ-induced neuronal degeneration (Miguel-Hidalgo et al., 2002) as well as working memory deficits (Yamada et al., 2005). These findings support the hypothesis that NMDA receptors play a central role in Aβ-induced neurotoxicity. In addition, the expression and activity of MMP-9 depend on the activation of NMDA receptors and are associated with the development of LTP (Meighan et al., 2006; Nagy et al., 2006). The activation of NMDA receptors promotes the development of dendritic

spines through MMP-mediated cell adhesion molecules (Tian et al., 2007). Together with our findings that Aβ treatment increased MMP-9 expression in primary cultured neurons, the activation and expression of MMP-9 are directly and/or indirectly regulated by Aβ through the activation of NMDA receptors in neuronal cells.

MMP-9 might be induced as a protection to destroy the plaques and amyloid fibrils. The growing list of proteases can degrade soluble Aβ in vitro, namely, neprilysin (Howell et al., 1995), insulin-degrading enzyme (Kurochkin and Goto, 1994), and MMP-9 (Yan et al., 2006), suggesting a role for these proteases in regulating endogenous basal levels of Aβ in vivo. Notably, MMP-9 was reported to cleave insoluble Aβ in vitro (Yan et al., 2006). The view of the function of MMPs in the long-lasting synaptic plasticity is expanding, and evidence suggests that MMP-9 is up-regulated and becomes proteolytically active selectively during the maintenance phase of LTP at CA3-CA1 synapses in the hippocampus (Nagy et al., 2006), and similar findings have been recently made in rat prefrontal cortex. These reports suggest that MMPs function in cellular processes that contribute to learning and memory. Therefore, although we assumed that MMP inhibitors potentiate the Aβ-induced cognitive dysfunction and neurotoxicity, our findings do not support such an assumption. In Supplemental Fig. 3, we showed that MMP inhibitor treatment ameliorated Aβ-induced impairment of recognition memory, suggesting that the transient increase in hippocampal MMP-9 activity is functionally associated with the development of Aβ-induced cognitive deficits. The findings made with a pharmacological inhibitor were further supported by the result that the intracerebroventricular injection of Aβ1-40 impaired recognition memory in wild-type but not MMP-9(−/−) mice. Accordingly, even if MMP-9 can degrade Aβ/plaques, it may randomly and nonselectively destroy the extracellular matrix and neural membranes, leading to neuronal dysfunction and cognitive impairment.

Recent evidence has linked MMPs to various pathological conditions in the central nervous system, including ischemia, multiple sclerosis, Parkinson's disease, and malignant glioma. This implies that, in addition to its known function to degrade extracellular macromolecules, MMP may serve as a mediator that leads to apoptotic and/or necrotic cell death. In fact, recent studies indicate that MMP-9 has direct neurotoxic effects. Jourquin et al. (2003) demonstrated the increased release and activity of MMP-9 after stimulation with neurotoxic kainate in organotropic cultures and reduced neuronal cell death by the inhibition of MMP-9. Conversely, incubation with recombinant MMP-9 induced neuron death in the organotropic cultures. Alternatively, MMP-3 is reported to play a major role in degenerative human brain disorders such as Parkinson' disease (Kim et al., 2005). In the present study, MMP inhibitor II, reported to be highly selective for MMP-2 and MMP-9 (Tamura et al., 1998), blocked the Aβ-induced release of LDH, indicating that MMP-9 is crucial in Aβ-induced neuronal cell death. Genetic evidence also showed that Aβ-induced neurotoxicity was markedly reduced in primary cultured cortical neurons from MMP-9(−/−) mice compared with those from wild-type mice (Fig. 7). However, there is a report that GM6001, a broad-spectrum metalloproteinase inhibitor, acts synergistically with Aβ to enhance neurotoxicity in cultured neurons (Ethell et al., 2002). The discrepancy may reflect differences in the cell death assay and the specificity of inhibitors used because GM6001 can inhibit all MMPs and a-disintegrin-and-a-metalloproteinases.

In conclusion, we have demonstrated for the first time that MMP-9 activity and protein expression are transiently increased in the hippocampus by the intracerebroventricular injection of Aβ25-35, Aβ1−40, and Aβ1−42. We hypothesize that Aβ-induced secondary dysfunction such as MMP activation, could result in learning deficits by impairing synaptic function in the hippocampus. The present findings highlight the contribution of neural/glial MMP-9 to Aβ-induced neurotoxicity and cognitive impairment and support the case for highly selective MMP-9 inhibitors that could reduce deleterious proteolytic activity and neuronal death. Thus, specific inhibitors of MMP-9 may have therapeutic potential for the treatment of AD.

References

Alkam T, Nitta A, Mizoguchi H, Itoh A, and Nabeshima T (2007) A natural scavenger of peroxynitrites, rosmarinic acid, protects against impairment of memory induced by Abeta(25-35). *Behav Brain Res* **180:**139–145.

Asahina M, Yoshiyama Y, and Hattori T (2001) Expression of matrix metalloproteinase-9 and urinary-type plasminogen activator in Alzheimer's disease brain. *Clin Neuropathol* **20:**60–63.

Backstrom JR, Lim GP, Cullen MJ, and Tökés ZA (1996) Matrix metalloproteinase-9 (MMP-9) is synthesized in neurons of the human hippocampus and is capable of degrading the amyloid-beta peptide (1-40). *J Neurosci* **16:**7910–7919.

Deb S and Gottschall PE (1996) Increased production of matrix metalloproteinases in enriched astrocyte and mixed hippocampal cultures treated with beta-amyloid peptides. *J Neurochem* **66:**1641–1647.

Deb S, Wenjun Zhang J, and Gottschall PE (2003) Beta-Amyloid induces the production of active, matrix-degrading proteases in cultured rat astrocytes. *Brain Res* **970:**205–213.

Ethell DW, Kinloch R, and Green DR (2002) Metalloproteinase shedding of Fas ligand regulates beta-amyloid neurotoxicity. *Curr Biol* **12:**1595–1600.

Galardy RE, Cassabonne ME, Giese C, Gilbert JH, Lapierre F, Lopez H, Schaefer ME, Stack R, Sullivan M, and Summers B (1994) Low molecular weight inhibitors in corneal ulceration. *Ann N Y Acad Sci* **732:**315–323.

Hardy J and Selkoe DJ (2002) The amyloid hypothesis of Alzheimer's disease: progress and problems on the road to therapeutics. *Science* **297:**353–356.

Harkany T, Abrahám I, Timmerman W, Laskay G, Tóth B, Sasvári M, Kónya C, Sebens JB, Korf J, Nyakas C, et al. (2000) Beta-Amyloid neurotoxicity is mediated by a glutamate-triggered excitotoxic cascade in rat nucleus basalis. *Eur J Neurosci* **12:**2735–2745.

Howell S, Nalbantoglu J, and Crine P (1995) Neutral endopeptidase can hydrolyze beta-amyloid(1-40) but shows no effect on beta-amyloid precursor protein metabolism. *Peptides* **16:**647–652.

Jourquin J, Tremblay E, Décanis N, Charton G, Hanessian S, Chollet AM, Le Diguardher T, Khrestchatisky M, and Rivera S (2003) Neuronal activity-dependent increase of net matrix metalloproteinase activity is associated with MMP-9 neurotoxicity after kainate. *Eur J Neurosci* **18:**1507–1517.

Kim YS, Kim SS, Cho JJ, Choi DH, Hwang O, Shin DH, Chun HS, Beal MF, and Joh TH (2005) Matrix metalloproteinase-3: a novel signaling proteinase from apoptotic neuronal cells that activates microglia. *J Neurosci* **25:**3701–3711.

Kurochkin IV and Goto S (1994) Alzheimer's beta-amyloid peptide specifically interacts with and is degraded by insulin degrading enzyme. *FEBS Lett* **345:**33–37.

Lo EH, Wang X, and Cuzner ML (2002) Extracellular proteolysis in brain injury and inflammation: role for plasminogen activations and matrix metalloproteinases. *J Neurosci Res* **69:**1–9.

Malm T, Ort M, Tähtivaara L, Jukarainen N, Goldsteins G, Puoliväli J, Nurmi A, Pussinen R, Ahtoniemi T, Miettinen TK, et al. (2006) Beta-Amyloid infusion results in delayed and age-dependent learning deficits without role of inflammation or beta-amyloid deposits. *Proc Natl Acad Sci U S A* **103:**8852–8857.

Miguel-Hidalgo JJ, Alvarez XA, Cacabelos R, and Quack G (2002) Neuroprotection by memantine against neurodegeneration induced by beta-amyloid(1-40). *Brain Res* **958:**210–221.

Meighan SE, Meighan PC, Choudhury P, Davis CJ, Olson ML, Zornes PA, Wright JW, and Harding JW (2006) Effects of extracellular matrix-degrading proteases matrix metalloproteinases 3 and 9 on spatial learning and synaptic plasticity. *J Neurochem* **96:**1227–1241.

Mizoguchi H, Takuma K, Fukakusa A, Ito Y, Nakatani A, Ibi D, Kim HC, and Yamada K (2008) Improvement by minocycline of methamphetamine-induced impairment of recognition memory in mice. *Psychopharmacology (Berl)* **196:**233–241.

Mizoguchi H, Yamada K, Mouri A, Niwa M, Mizuno T, Noda Y, Nitta A, Itohara S, Banno Y, and Nabeshima T (2007a) Role of matrix metalloproteinase and tissue inhibitor of MMP in methamphetamine-induced behavioral sensitization and reward: implications for dopamine receptor down-regulation and dopamine release. *J Neurochem* **102:**1548–1560.

Mizoguchi H, Yamada K, Niwa M, Mouri A, Mizuno T, Noda Y, Nitta A, Itohara S, Banno Y, and Nabeshima T (2007b) Reduction of methamphetamine-induced sensitization and reward in matrix metalloproteinase-2 and -9-deficient mice. *J Neurochem* **100:**1579–1588.

Mouri A, Noda Y, Hara H, Mizoguchi H, Tabira T, and Nabeshima T (2007) Oral vaccination with a viral vector containing Abeta cDNA attenuates age-related Abeta accumulation and memory deficits without causing inflammation in a mouse Alzheimer model. *FASEB J* **21:**2135–2148.

Muir EM, Adcock KH, Morgenstern DA, Clayton R, von Stillfried N, Rhodes K, Ellis C, Fawcett JW, and Rogers JH (2002) Matrix metalloproteases and their inhibitors are produced by overlapping populations of activated astrocytes. *Brain Res Mol Brain Res* **100:**103–117.

Nagy V, Bozdagi O, Matynia A, Balcerzyk M, Okulski P, Dzwonek J, Costa RM, Silva AJ, Kaczmarek L, and Huntley GW (2006) Matrix metalloproteinase-9 is required for hippocampal late-phase long-term potentiation and memory. *J Neurosci* **26:** 1923–1934.

Selkoe DJ and Schenk D (2003) Alzheimer's disease: molecular understanding predicts amyloid-based therapeutics. *Annu Rev Pharmacol Toxicol* **43:**545–584.

Snyder EM, Nong Y, Almeida CG, Paul S, Moran T, Choi EY, Nairn AC, Salter MW, Lombroso PJ, Gouras GK, et al. (2005) Regulation of NMDA receptor trafficking by amyloid-beta. *Nat Neurosci* **8:**1051–1058.

Szklarczyk A, Lapinska J, Rylski M, McKay RD, and Kaczmarek L (2002) Matrix metalloproteinase-9 undergoes expression and activation during dendritic remodeling in adult hippocampus. *J Neurosci* **22:**920–930.

Takuma K, Yan SS, Stern DM, and Yamada K (2005a) Mitochondrial dysfunction, endoplasmic reticulum stress, and apoptosis in Alzheimer's disease. *J Pharmacol Sci* **97:**312–316.

Takuma K, Yao J, Huang J, Xu H, Chen X, Luddy J, Trillat AC, Stern DM, Arancio O, and Yan SS (2005b) ABAD enhances Abeta-induced cell stress via mitochondrial dysfunction. *FASEB J* **19:**597–598.

Tamura Y, Watanabe F, Nakatani T, Yasui K, Fuji M, Komurasaki T, Tsuzuki H, Maekawa R, Yoshioka T, Kawada K, et al. (1998) Highly selective and orally active inhibitors of type IV collagenase (MMP-9 and MMP-2): *N*-sulfonylamino acid derivatives. *J Med Chem* **41:**640–649.

Tian L, Stefanidakis M, Ning L, Van Lint P, Nyman-Huttunen H, Libert C, Itohara S, Mishina M, Rauvala H, and Gahmberg CG (2007) Activation of NMDA receptors promotes dendritic spine development through MMP-mediated ICAM-5 cleavage. *J Cell Biol* **178:**687–700.

Tran MH, Yamada K, Olariu A, Mizuno M, Ren XH, and Nabeshima T (2001) Amyloid beta-peptide induces nitric oxide production in rat hippocampus: association with cholinergic dysfunction and amelioration by inducible nitric oxide synthase inhibitors. *FASEB J* **15:**1407–1409.

Van den Steen PE, Dubois B, Nelissen I, Rudd PM, Dwek RA, and Opdenakker G (2002) Biochemistry and molecular biology of gelatinase B or matrix metalloproteinase-9 (MMP-9). *Crit Rev Biochem Mol Biol* **37:**375–536.

Wang D, Noda Y, Zhou Y, Mouri A, Mizoguchi H, Nitta A, Chen W, and Nabeshima T (2007) The allosteric potentiation of nicotinic acetylcholine receptors by galantamine ameliorates the cognitive dysfunction in beta amyloid25-35 i.c.v.-injected mice: involvement of dopaminergic systems. *Neuropsychopharmacology* **32:**1261–1271.

Wang J and Tsirka SE (2005) Neuroprotection by inhibition of matrix metalloproteinases in a mouse model of intracerebral haemorrhage. *Brain* **128:**1622–1633.

Wenk GL (2006) Neuropathologic changes in Alzheimer's disease: potential targets for treatment. *J Clin Psychiatry* **67:**3–7.

Yamada K and Nabeshima T (2000) Animal models of Alzheimer's disease and evaluation of anti-dementia drugs. *Pharmacol Ther* **88:**93–113.

Yamada K, Takayanagi M, Kamei H, Nagai T, Dohniwa M, Kobayashi K, Yoshida S, Ohhara T, Takuma K, and Nabeshima T (2005) Effects of memantine and donepezil on amyloid beta-induced memory impairment in a delayed-matching to position task in rats. *Behav Brain Res* **162:**191–199.

Yamada K, Tanaka T, Han D, Senzaki K, Kameyama T, and Nabeshima T (1999) Protective effects of idebenone and alpha-tocopherol on beta-amyloid-(1-42)-induced learning and memory deficits in rats: implication of oxidative stress in beta-amyloid-induced neurotoxicity in vivo. *Eur J Neurosci* **11:**83–90.

Yan P, Hu X, Song H, Yin K, Bateman RJ, Cirrito JR, Xiao Q, Hsu FF, Turk JW, Xu J, et al. (2006) Matrix metalloproteinase-9 degrades amyloid-beta fibrils in vitro and compact plaques in situ. *J Biol Chem* **281:**24566–24574.

Yong VW, Power C, Forsyth P, and Edwards DR (2001) Metalloproteinases in biology and pathology of the nervous system. *Nat Rev Neurosci* **2:**502–511.

Address correspondence to: Dr. Kiyofumi Yamada, Department of Neuropsychopharmacology and Hospital Pharmacy, Nagoya University Graduate School of Medicine, Showa-ku, Nagoya 466-8560, Japan. E-mail: kyamada@med.nagoya-u.ac.jp