Aβ42-to-Aβ40- and Angiotensin-converting Activities in Different Domains of Angiotensin-converting Enzyme*

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Amyloid β-protein 1–42 (Aβ42) is believed to play a causative role in the development of Alzheimer disease (AD), although it is a minor part of Aβ. In contrast, Aβ40 is the predominant secreted form of Aβ and recent studies have suggested that Aβ40 has neuroprotective effects and inhibits amyloid deposition. We have reported that angiotensin-converting enzyme (ACE) converts Aβ42 to Aβ40, and its inhibition enhances brain Aβ42 deposition (Zou, K., Yamaguchi, H., Akatsu, H., Sakamoto, T., Ko, M., Mizoguchi, K., Gong, J. S., Yu, W., Yamamoto, T., Kosaka, K., Yanagisawa, K., and Michikawa, M. (2007) J. Neurosci. 27, 8628–8635). ACE has two homologous domains, each having a functional active site. In the present study, we identified the domain of ACE, which is responsible for converting Aβ42 to Aβ40. Interestingly, Aβ42-to-Aβ40-converting activity is solely found in the N-domain of ACE and the angiotensin-converting activity is found predominantly in the C-domain of ACE. We also found that the N-linked glycosylation is essential for both Aβ42-to-Aβ40- and angiotensin-converting activities and that unglycosylated ACE rapidly degraded. The domain-specific converting activity of ACE suggests that ACE inhibitors could be designed to specifically target the angiotensin-converting C-domain, without inhibiting the Aβ42-to-Aβ40-converting activity of ACE or increasing neurotoxic Aβ42.

Angiotensin-converting enzyme (ACE)4 plays a key role in the renin-angiotensin system (RAS), which is involved in the long-term regulation of blood pressure and blood volume in the human body. Recent genetic, pathologic, and biochemical studies have associated ACE with onset of Alzheimer disease (AD) (1, 2). The I allele of the ACE gene, which results in a reduced serum ACE level, has been demonstrated to be associated with AD (3–5). Hypertension is a risk factor for AD and ACE inhibitors for treatment of hypertension were shown to be the only drug class among the antihypertensives to potentially be associated with a slight increased incidence of AD (adjusted hazard ratio 1.13) (6, 7). A mechanistic link between ACE and AD was suggested when ACE was shown to degrade Aβ40 and Aβ42 (8, 9). Overexpression of Aβ40 in transgenic mice does not cause brain amyloid deposition, the major pathologic hallmark of AD, whereas expression of Aβ42 is shown to be essential for amyloid deposition (10, 11). In addition, Aβ40 has an inhibitory effect on amyloid deposition in vitro and in vivo and has neuroprotective effects (12–14). These lines of evidence suggest that converting Aβ42 to Aβ40 may be a potential strategy for development of an AD therapy. In our previous study, we identified ACE as an Aβ42-to-Aβ40-converting (Aβ-converting) enzyme and showed that ACE inhibitor enhances brain Aβ42 deposition in transgenic mice (15). Clarifying the molecular base of ACE domain-specific enzymatic activity on Aβ42 to Aβ40 conversion, Aβ degradation, and angiotensin conversion emerges to be important for development of a strategy for hypertension and AD treatment.

ACE is a type I integral membrane glycoprotein, and there are two isoforms of ACE in mammals that arise from the use of alternative promoters in a single gene: somatic ACE and testicular ACE. ACE also has one mammalian relative, ACE2, which consists of a single active site domain that, by sequence comparison, more closely resembles the N-domain than the C-domain of somatic ACE. ACE converts angiotensin I to angiotensin II, a potent vasoconstrictor, and inactivates bradykinin, a vasodilator (16). Given the central role ACE plays in regulation of blood pressure, ACE inhibitors are widely used for the treatment of hypertension in the elderly population. ACE also hydrolyzes a wide range of polypeptide substrates, including substance P, luteinizing hormone-releasing hormone, acetylser-Asp-Lys-Pro (AcSDKP), and neurotensin (16). The mammalian somatic ACE contains two homologous domains, the N-terminal domain (N-domain) and C-terminal domain (C-domain), each bearing a zinc-dependent active site. The pres-
ence of two active sites in ACE has stimulated many attempts to establish whether they differ in function. For example, AcSDKP, a peptide suggested to inhibit bone marrow maturation, is found to be preferentially cleaved by the N-domain of ACE *in vitro* (17). In contrast, the ACE C-domain is demonstrated to be the main site of angiotensin I cleavage *in vivo* (18).

The N-linked glycosylation of testicular ACE, a homologue of the somatic ACE N-domain, is essential for its enzymatic activity and for preventing degradation (19).

In our current study, we determined the contributions of each ACE domain, toward Aβ42-to-Aβ40- and/or angiotensin-converting activity. We postulated that the dipeptidyl carboxypeptidase activity of ACE, which converts angiotensin I to angiotensin II and Aβ42 to Aβ40, is located in its C-domain. Surprisingly, we found that the Aβ42-to-Aβ40-converting activity is specifically in the N-domain of ACE, and the angiotensin-converting activity is predominantly in the C-domain of ACE. We also found that both Aβ42-to-Aβ40- and angiotensin-converting activities require the N-linked glycosylation of ACE. The finding of domain-specific Aβ42-to-Aβ40-converting activity of ACE may help design a domain-specific ACE inhibitor for treatment of hypertension, without inhibiting the N-domain-specific Aβ42-to-Aβ40-converting activity of ACE.

**EXPERIMENTAL PROCEDURES**

**Truncated ACE Expression and Purification**—Expression and purification of ACE recombinant proteins were carried out as described previously (20). Mutated ACE cDNAs containing two active domains (F-ACE) or only the N-terminal active domain or C-terminal active domain (N-ACE or C-ACE) were cloned into pcDNA3.1(-) vectors (Invitrogen). Six histidine residues were introduced at the C-terminal end of each cDNA. The C-terminal transmembrane domain was removed from all of the recombinant ACE proteins to allow them to be secreted into the culture medium. COS7 cells were grown in Dulbecco’s modified Eagle’s medium (DMEM) containing 10% fetal bovine serum. Transfections of the ACE pcDNA3.1(-) vectors in COS7 cells were performed using Lipofectamine 2000 (Invitrogen), and COS7 cells stably expressing F-, N-, and C-ACE were transiently transfected with the vectors bearing ACE full-length expression vectors bearing the catalytically inactive full-length ACE were kindly provided by Dr. Dennis J. Selkoe (9). The two ACE zinc metalloprotease active site glutamates (amino acids 362 in the somatic ACE N-domain, is essential for its enzymatic activity and for preventing degradation (19).

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**ACE Activity Assay**—F-ACE, N-ACE, and C-ACE were dialyzed in 50 mM HEPES, 50 mM NaCl, 1 mM ZnCl₂, pH 7.5, and their activities against the synthetic substrate N-hippuryl-l-histidyl-l-leucine (Hip-His-Leu) were determined using an ACE colorimetric kit (Buhlmann Laboratories, Schonenbuch, Switzerland). 10 μl of ACE proteins at a concentration of 0.5 μM were mixed and incubated with ACE substrate at 37 °C. The reaction time was 15 min. All samples were measured in triplicate.

**Mass Spectrometry Analysis**—Purified F-ACE, N-ACE, or C-ACE was incubated with 80 μM Aβ42 at 37 °C for 2 h. Captopril (10 μM) was added to stop digestion, and the sample was frozen in −80 °C until use. The samples were mixed with 3,5-dimethoxy-4-hydroxycinnamic acid (Wako, Japan) as a matrix, and then subjected to matrix-assisted laser desorption ionization-time of flight-mass spectrometry (MALDI-TOF-MS) (AXIMA-CFR, SHIMADZU, Kyoto, Japan) to detect the generation of Aβ40 and other Aβ fragments. The same amount of F-ACE, N-ACE, C-ACE, or Aβ42 incubated alone under the same conditions as described above was used as control.

**Expression of ACE Active Site Mutants and Determining Their Domain-specific Activities**—The pcDNA5/FRT expression vectors bearing the catalytically inactive full-length ACE were kindly provided by Dr. Dennis J. Selkoe (9). The two ACE zinc metalloprotease active site glutamates (amino acids 362 in the N-domain and 960 in the C-domain) were changed to aspartate. Mouse embryonic fibroblasts at 90% confluence were transiently transfected with the vectors bearing ACE full-length protein with active site mutations using Lipofectamine 2000 (Invitrogen). After 48 h, the cells were lysed in in 50 mM Tris/HCl (pH 7.5) containing 0.5% Nonidet P-40, and nuclei and cell debris was pelleted at 10,000 × g for 10 min at 4 °C. To assay ACE activity, 5 μg of protein of cell lysate was added to stop digestion, and the conversion of Aβ40 from Aβ42 was detected by Western blot.

**Deglycosylation of ACE Proteins**—To assess the type of glycosylation of human kidney ACE and recombinant ACE proteins, the ACE proteins were treated with PNGase F, O-glycanase, or sialidase A using an enzymatic deglycosylation kit according to the manufacturer’s instructions (PROzyme, San Leandro, CA). To evaluate the enzymatic activities of deglycosylated ACE proteins, non-denaturing protocol was used, and ACE proteins

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**Western Blot Analysis and Determining Conversion of Aβ42 to Aβ40**—COS7 cells were lysed in radioimmunoprecipitation assay buffer (10 mM Tris/HCl (pH 7.5), 150 mM NaCl, 1% Nonidet P-40, 0.1% sodium dodecyl sulfate (SDS), and 0.2% sodium deoxycholate, containing a protease inhibitor mixture (Roche Applied Science)). The expression of ACE recombinant proteins was detected by Western blotting using a polyclonal anti-ACE antibody (R&D). Aβ1–42 (Peptide Institute) was freshly dissolved in 0.1% NH₄H₂O at 200 μM for each experiment. 80 μl of F-, N-, and C-ACE at a concentration of 0.5 μM were mixed with synthetic Aβ42 to a final concentration of 40 μM and incubated at 37 °C. 10 μl of the mixture was subjected to SDS-PAGE and blotted on a nitrocellulose membrane. To enhance the reactivity to an anti-Aβ40 antibody, the membrane was boiled in PBS for 3 min after blotting, probed with an anti-Aβ40 monoclonal antibody (1A10) (IBL), and visualized with SuperSignal (Pierce). Because of the high level of exogenous Aβ42, the membrane was not boiled before the reaction with a polyclonal anti-Aβ42 antibody. The quantification of Aβ40 generation and Aβ42 degradation was carried out using Image J 1.41 software (NIH).

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were deglycosylated at 37 °C for 1 h. The non-deglycosylated ACE proteins were mixed with the same incubation buffer provided by the manufacturer and incubated except that glycosidases were not added.

RESULTS

ACE N-domain, but Not C-domain, Converts Aβ42 to Aβ40—

To explore which domain of ACE has Aβ42-to-Aβ40-converting activity, we prepared 3 kinds of recombinant ACE proteins, which were transfected into COS7 cells. F-ACE contains both the N-domain and C-domain active sites. N-ACE contains only the N-terminal active site, and C-ACE only contains the C-terminal active site. All three kinds of mutated ACE were fused with a 6-histidine tag at the C terminus and a signal peptide at the N terminus. B, COS7 cells transfected with empty vector or cells stably expressing F-ACE, N-ACE, or C-ACE were lysed in radioimmune precipitation assay buffer. Western blots of 20 µg of total protein from the cells or 2 µg of ACE isolated from the culture medium were probed with a polyclonal anti-ACE antibody. C, ACE activity was measured by incubating 0.5 µM F-ACE, N-ACE, or C-ACE with the substrate Hip-His-Leu for 15 min at 37 °C. N-ACE has markedly reduced ACE activity compared with C-ACE. Values represent the means ± S.E.; n = 3; *, p < 0.001, Bonferroni/Dunn test. D, specificities of monoclonal anti-Aβ40 (1A10) and polyclonal anti-Aβ42 antibodies were confirmed by Western blot of 0.1 µg of Aβ40 and Aβ42. E, F-, N-, and C-ACE were mixed with synthetic Aβ42 and incubated at 37 °C for 0.5, 1, or 2 h. Western blots of the mixture were probed with anti-Aβ40 and anti-Aβ42 antibodies. In contrast to the ACE activity, the Aβ42-to-Aβ40-converting activity was solely detected in N-ACE. F, generation of Aβ40 and the degradation of Aβ42 were determined by densitometry.

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ACE N-domain Converts Aβ42 to Aβ40
two antibodies was not found (Fig. 1D). Unexpectedly, in contrast to the angiotensin-converting activity, the Aβ42-to-Aβ40-converting activity was found in F-ACE and N-ACE, but not in C-ACE, indicating that N-domain of ACE has the Aβ42-to-Aβ40-converting activity. Although C-ACE showed a similar Aβ42-degrading activity compared with F-ACE and N-ACE, it did not generate Aβ40 from Aβ42 (Fig. 1E). Incubation of C-ACE with Aβ42 up until 16 h did not generate Aβ40 (data not shown). F-ACE generated more Aβ40 from Aβ42 than N-ACE at the time points of 0.5 and 1 h, whereas at the time point of 2 h F-ACE and N-ACE generated similar amounts of Aβ40 (Fig. 1, E and F). F-ACE had similar activities compared with native human kidney ACE regarding the Aβ42-to-Aβ40-converting activity and the Hip-His-Leu-degrading activity (Figs. 1E and 4C and data not shown).

To determine other products other than Aβ40 that were generated by ACE from Aβ42 and to confirm the result from Western blot, we performed mass spectrometry analysis. Consistent with our immunological studies, a peak corresponding to Aβ1–40 was detected in the F-ACE- and N-ACE-digested samples; in addition, F-ACE and N-ACE also generated peaks corresponding to Aβ1–33, Aβ1–28, Aβ1–24, and Aβ1–21 from Aβ42, whereas Aβ1–40 was not formed by C-ACE. However, C-ACE generated four other Aβ fragments, Aβ1–33, Aβ1–28, Aβ1–24, and Aβ1–21 (Fig. 2A). Mass spectrometry analysis for incubated F-ACE, N-ACE, or C-ACE alone did not show any Aβ peptide signal, and synthetic Aβ42 only showed one peak with a mass at 4514, which matched the predicted mass of Aβ1–42 (Fig. 2B and data not shown). These results from mass spectrometry confirmed that the Aβ42-to-Aβ40-converting activity is restricted to the ACE N-domain.

N-domainInactive ACE Mutant Loses Aβ42-to-Aβ40-converting Activity—Three ACE mutants were generated by site-directed mutagenesis to change the active site sequence HEMGH to HDMGH in N-, C-, or both N- and C-domain. The N-domain active site was inactivated by mutating glutamate 362 to aspartate (termed E362D), and the C-domain active site was similarly inactivated by mutating glutamate 960 to aspartate (termed E960D). E362/960D has double mutations converting Activity

ACE N-domain Converts Aβ42 to Aβ40

FIGURE 2. MALDI-TOF-MS analysis for Aβ42 degradation by F-ACE, N-ACE, or C-ACE. A, Aβ42 (80 μM) was incubated with 0.5 μM purified F-ACE, N-ACE, or C-ACE at 37 °C for 2 h, then captopril (10 μM) was added after incubation to stop the digestion. 1 μl of the mixture was subjected to MALDI-TOF-MS analysis. F-ACE and N-ACE generated Aβ1–40, whereas C-ACE did not. B, 1 μl of F-ACE, N-ACE, or C-ACE alone incubated at 37 °C for 2 h was subjected to MALDI-TOF-MS analysis, and a peptide signal was not detected. wtACE, whereas E362D and E362/960D without N-domain activity did not convert Aβ42 to Aβ40 (Fig. 3D, middle panel).

N-Glycosylation Is Essential for Aβ42-to-Aβ40- and Angiotensin-converting Activities—ACE is a glycoprotein, and the N-linked glycosylation of testicular ACE has been shown to be essential for its angiotensin-converting activity. Human ACE has 17 putative Asn(X)Ser/Thr N-linked glycosylation sites distributed throughout both the N-domain and C-domain (21). To determine the role of glycosylation of ACE in its enzymatic activities and to compare the glycosylation of natural human ACE with that of recombinant F-ACE, N-ACE, and C-ACE, we examined the type of glycosylation of these ACE proteins by the treatment with PNGase F, O-glycanase, and sialidase A. Treatment with PNGase F, O-glycanase, and sialidase A remarkably reduced the molecular weight of human kidney ACE, F-ACE, N-ACE, and C-ACE (Fig. 4A, lanes 1 and 2). Removal of N-linked glycosylation using PNGase F alone produced similar molecular weight shifts, whereas O-glycanase did not produce any shift in ACE size (Fig. 4A, lanes 3 and 4). The sensitivity of N-ACE to PNGase F indicates that N-ACE is modified by N-linked glycosylation. All the ACE proteins showed a slight decrease in the molecular weight after sialidase A digestion,
indicating the sialylation of their N-glycans (Fig. 4A, lane 5). These results suggest that the glycosylation type of natural human kidney ACE and recombinant ACE proteins produced by COS7 cells are identical. Because ACE is modified by N-linked glycosylation and O-linked glycosylation was not detected, we used PNGase to remove its N-glycans and studied the ACE activity. As expected, PNGase-treated human kidney ACE showed a 96% reduced ACE activity in degradation of Hip-His-Leu compared with untreated ACE (Fig. 4B).

To determine the role of N-linked glycosylation of ACE in its Aβ42-to-Aβ40-converting activity, we incubated Aβ42 with PNGase F-treated or untreated human kidney ACE and examined Aβ40 generation by Western blot. Deglycosylated human kidney ACE showed a decreased molecular mass at ~150 kDa and was degraded by itself after 2 h of incubation. After incubation for 16 h, ~150-kDa deglycosylated ACE was completely degraded (Fig. 4C, upper panel). Aβ40 was generated from Aβ42 by ACE after incubating the mixture of Aβ42 and ACE for 15 min. The level of Aβ40 increased in a time-dependent manner and reached a peak after incubation for 2 h, whereas deglycosylated ACE did not generate Aβ40 from Aβ42, although it showed a similar Aβ42-degrading activity compared with non-deglycosylated ACE (Fig. 4C, middle and bottom panels). This glycosylation-required Aβ42-to-Aβ40-converting activity was also confirmed in recombinant ACE proteins. PNGase F-deglycosylated F-ACE, N-ACE, and C-ACE have similar Aβ42-degrading activity. However, deglycosylated F-ACE and N-ACE failed to generate Aβ40 from Aβ42, suggesting that the N-linked glycosylation in the ACE N-domain is essential for its Aβ42-to-Aβ40-converting activity (Fig. 4D). Sialidase A treatment did not change the Aβ42-to-Aβ40-converting activity and the ACE activity of human kidney ACE, indicating that sialylation is not required for its activities (data not shown).

Captopril and Enalaprilat Showed Different IC_{50} on Aβ42-to-Aβ40-converting Activity—The feature of ACE inhibitors has been well studied in terms of their angiotensin-converting inhibitory effect. To explore whether ACE inhibitors differentially inhibit the Aβ42-to-Aβ40-converting activity, we determined the IC_{50} of captopril, perindopril, lisinopril, and enalaprilat toward the angiotensin- and Aβ42-to-Aβ40-converting activity of F-ACE. All four ACE inhibitors showed a similar IC_{50} on the inhibition of angiotensin-converting activity of F-ACE, whereas enalaprilat exhibited a 10-fold lower IC_{50} (0.003–0.01 μM) on Aβ42-to-Aβ40-converting activity than captopril (0.03–0.1 μM) (Table 1).

**FIGURE 3.** Site-directed mutated ACE proteins exhibit domain-specific Aβ42-to-Aβ40- and angiotensin-converting activity. A schematic representation of human ACE and the mutant positions. The two ACE zinc metalloprotease active site glutamates (amino acids 362 in the N-domain and 960 in the C-domain) were changed to aspartates. B, fibroblasts were transiently transfected with empty vector, wtACE or mutant ACE plasmids and the expression of ACE proteins was detected by Western blotting using a polyclonal antianti-ACE antibody. C, ACE activity was measured by incubating 5 μg of protein of cell lysate with the substrate Hip-His-Leu for 10 min at 37 °C. ACE activity in cell lysate was clearly detected in wtACE and E362D. C-domain inactive ACE protein, E960D, showed an extremely low ACE activity; and double mutants in both domains of ACE, E362D/960D, did not show ACE activity. ACE activity was clearly inhibited by captopril (1 μM) treatment. D, ACE in cell lysate (4 mg of protein) from each transfected cell line was immunoprecipitated by 5 μg of polyclonal anti-ACE antibody and 100 μl of protein G-Sepharose. Immunoprecipitated ACE was then incubated with synthetic Aβ42 and the generation of Aβ40 was detected by Western blotting. SP, signal peptide; TM, transmembrane.

**DISCUSSION**

Most mammalian tissues contain ACE with two catalytic domains. Evolutionary conservation of the ACE N- and C-domains suggests important distinct functions of these domains. Recent genetic studies have associated the I allele of the ACE gene, which results in a reduced serum ACE level, with onset of AD (1, 3). We have shown previously that ACE converts Aβ42 to Aβ40, and its inhibition predominantly enhances brain Aβ42 deposition (15). To investigate which domain of ACE is responsible for Aβ42-to-Aβ40-converting activity and whether ACE inhibitors inhibit this activity, we generated three kinds of ACE proteins, containing both N- and C-domains or containing either single active domain. We also used selective site-directed mutagenesis of ACE to study the domain-specific activity of full-length ACE. The present study shows that the Aβ42-to-Aβ40- and angiotensin-converting activities were located in different ACE domains and that N-linked glycosylation was essential for the two ACE enzymatic activities. The N-domain of ACE clearly showed an Aβ42-to-Aβ40-converting activity, whereas it has an extremely low angiotensin-converting activity. In contrast, the C-domain of ACE showed angiotensin-converting activity, whereas the Aβ42-to-Aβ40-converting activity was not detected in this domain.

In a cellular context, both the N-domain and C-domains of ACE are able to degrade Aβ40 and Aβ42 (9). In our studies, we also found that the N- and C-domains were indistinguishable as regarding degrading Aβ42, suggesting that both N- and C-domains of ACE have endopeptidase activity for Aβ42. In the overall scheme of Aβ42 processing, the full-length ACE cleaving into many fragments may be important for therapeutic treatment of AD. We showed that Aβ40, but not Aβ41, was generated from Aβ42 (Fig. 2A). However, the Aβ42-to-Aβ40-converting activity was solely found in the N-domain of ACE (Figs. 1, 2, and 3). These results suggest that the dipeptidyl carboxypeptidase activity converting Aβ42 to Aβ40 is restricted to its N-domain. The N-domain specific dipeptidyl activity was
Characterization of ACE glycosylation and role of the glycosylation in ACE activity and Aβ42-to-Aβ40-converting activity. A, 5 μg of purified human kidney ACE, F-ACE, N-ACE, and C-ACE were deglycosylated with 1 μl of PNGase F, O-glycanase, and/or sialidase A for 1 h at 37 °C. PNGase F alone was able to remove all glycosylation of ACE. B, ACE activity of PNGase F-deglycosylated human kidney ACE was measured immediately after deglycosylation using an ACE colorimetric kit. ACE activity was almost completely abolished by N-deglycosylation. C, 80 μl of human kidney ACE (0.5 μM) with or without N-deglycosylation was mixed with synthetic Aβ42 (40 μM) and incubated at 37 °C. 10 μl of the mixture were collected at various incubation time points and subjected to Western blot analysis. Deglycosylated ACE showed no Aβ42-to-Aβ40-converting activity, whereas the Aβ42-degrading activity remained. D, 40 μl of recombinant F-, N-, and C-ACE proteins (0.5 μM) were deglycosylated and mixed with Aβ42 and incubated at 37 °C for 1, 2, or 16 h. Aβ42-to-Aβ40-converting activity was not detected in either deglycosylated F-ACE or deglycosylated N-ACE, whereas all the deglycosylated ACE showed an Aβ42-degrading activity.

**TABLE 1**

ACE inhibitors inhibited Aβ42-to-Aβ40-converting activity with different IC₅₀

ACE activity of 10 μl of F-ACE (0.5 μM) was measured using an ACE colorimetric kit, and Aβ42-to-Aβ40-converting activity was measured by Western blotting and densitometry. 0, 0.003, 0.01, 0.03, 0.1, 0.3, 1, 3, 10 μM ACE inhibitors were added to determine the IC₅₀ for Aβ42-to-Aβ40-converting activity.

| ACE inhibitors   | ACE activity IC₅₀ | Aβ-converting activity IC₅₀* |
|------------------|------------------|----------------------------|
| Captopril        | 0.01–0.03        | 0.03–0.1                   |
| Enalaprilat      | 0.01–0.03        | 0.003–0.01                 |
| Lisinopril       | 0.01–0.03        | 0.01–0.03                  |
| Perindopril      | 0.03–0.1         | 0.01–0.03                  |

* Aβ-converting activity, Aβ42-to-Aβ40-converting activity.

also found in the degradation of AcSDKP, which is involved in the control of hematopoietic stem cell proliferation. The molecular basis in which the N-domain of ACE accesses AcSDKP and Aβ42 remains to be elucidated. The N- and C-domains of ACE have reduced Aβ42-to-Aβ40-converting activity and angiotensin-converting activity, respectively, compared with full domain ACE (Fig. 1, C and F), suggesting that each catalytic domain of ACE regulates the activity of the other, and both domains are required for normal substrate recognition and degradation. Mice with a selective inactivation of either the N- or C-domain of ACE were generated, and the C-domain was demonstrated to be the main site of angiotensin I cleavage (18, 22), which is consistent with our in vitro finding. However, the role of the N-domain of ACE toward Aβ42 to Aβ40 conversion in vivo needs to be addressed.

It has been previously reported that testicular ACE, the C-domain isof orm of ACE, without N-linked glycosylation has no enzyme activity and was rapidly degraded (19). We confirmed that deglycosylation of human kidney ACE abolished its angiotensin-converting activity, whereas the endopeptidase activity for degrading itself and Aβ42 was not affected. Moreover, the N-domain-specific Aβ42-to-Aβ40-converting activity was abolished by the deglycosylation, indicating that the N-linked glycosylation is also essential for maintaining the N-domain-specific enzymatic activity of ACE. Deglycosylated ACE was retained as an intact protein 30 min after deglycosylation. ACE activity and Aβ42-to-Aβ40-converting activity were clearly detected in non-deglycosylated ACE within 30 min (Fig. 4, B and C). Thus, the loss of ACE activity and Aβ42-to-Aβ40-converting activity of deglycosylated ACE may not result from its self-degradation, but likely result from the deglycosylation. These results suggest that N-linked glycosylation is required to maintain the ACE structure and its dipeptidyl carboxypeptidase activity in both N- and C-domains. Presenilins have been shown to be involved in the maturation of membrane proteins, whether presenilin mutants in familial AD affect ACE glycosylation and its Aβ42-to-Aβ40-converting activity need to be clarified in future (23). Finally, we showed that ACE inhibitors inhibited the N-domain-specific Aβ42-to-Aβ40-converting activity each with a different IC₅₀. Among the examined ACE inhibitors, enalaprilat has the strongest inhibitory effect on Aβ42-to-Aβ40-converting activity. This result may provide a mechanism underlying the finding that non-centrally active ACE inhibitors, such as enalaprilat, are associated with a greater risk of incident dementia (24).
our previous *in vivo* study, captopril treatment enhanced predominantly brain Aβ42 deposition in 17-month-old amyloid precursor protein (APP) transgenic mice and led to a tendency of increased brain Aβ42/40 ratio (15). Taking the anti-amyloid and antioxidant effects of Aβ40 into account, our findings suggest that ACE inhibitors could be designed specifically to target the C-domain of ACE without inhibiting its N-domain-specific Aβ42-to-Aβ40-converting activity.

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