Alzheimer’s Disease β-Amyloid Peptide Is Increased in Mice Deficient in Endothelin-converting Enzyme*

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The abnormal accumulation of β-amyloid (Aβ) in the brain is an early and invariant feature in Alzheimer’s disease (AD) and is believed to play a pivotal role in the etiology and pathogenesis of the disease. As such, a major focus of AD research has been the elucidation of the mechanisms responsible for the generation of Aβ. As with any peptide, however, the degree of Aβ accumulation is dependent not only on its production but also on its removal. In cell-based and in vitro models we have previously characterized endothelin-converting enzyme-1 (ECE-1) as an Aβ-degrading enzyme that appears to act intracellularly, thus limiting the amount of Aβ available for secretion. To determine the physiological significance of this activity, we analyzed Aβ levels in the brains of mice deficient for ECE-1 and a closely related enzyme, ECE-2. Significant increases in the levels of both Aβ40 and Aβ42 were found in the brains of these animals when compared with age-matched littermate controls. The increase in Aβ levels in the ECE-deficient mice provides the first direct evidence for a physiological role for both ECE-1 and ECE-2 in limiting Aβ accumulation in the brain and also provides further insight into the factors involved in Aβ clearance in vivo.

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The abbreviations used are: AD, Alzheimer’s disease; Aβ, β-amyloid; ECE, endothelin-converting enzyme; ET, endothelin; CHAPS, 3-[3-cholamidopropyl]dimethylammonio-1-propanesulfonic acid; ELISA, enzyme-linked immunosorbent assay; APP, Aβ precursor protein; CTF, C-terminal fragment.
**RESULTS**

To determine whether reduced ECE-1 activity would lead to increased Aβ levels in the brain, we compared the amount of Aβ in brains from ECE-1 (+/−) mice to that in wild-type littermate controls. The levels of both Aβ40 and Aβ42 were significantly increased in the mice with reduced ECE-1 activity (Fig. 1 and Table I). Although modest, these increases are in the range observed for some of the familial Alzheimer’s disease-linked mutations (29, 31–36) and are particularly noteworthy, as ECE-1 (+/−) mice have only an ∼27% reduction in ECE-1 activity (26 and data not shown). To determine whether the increase in Aβ concentration in the brains of these animals might be due to an increase in expression and/or processing of the Aβ precursor protein (APP), we examined the levels of APP and the C-terminal fragment of APP, which serves as the immediate precursor to Aβ (CTFβ). Consistent with the increase in Aβ concentration being a result of decreased degradation of Aβ, the levels of APP and CTFβ were unchanged in the ECE-1 (+/−) mice compared with their wild-type littermates (Fig. 2).

Given the catalytic similarity between ECE-1 and ECE-2 and that ECE-2 expression is greater in the nervous system than in other tissues, we also examined whether Aβ concentration is affected by the level of ECE-2 activity in the brain. ECE-2 (−/−) animals are viable and appear healthy (25). Thus we were able to analyze both ECE-2 (+/+) and ECE-2 (−/−) mice and compare the amount of Aβ in the brains of these animals to that found in littermate controls with normal ECE-2 levels. In these studies we found a significant elevation in the concentrations of both Aβ40 and Aβ42 in the brains of animals with reduced ECE activity compared with their control littermates (Fig. 1 and Table I). The concentration of Aβ in the brains of ECE-2 heterozygous mice was intermediate between that observed in the wild-type controls and the complete nulls, suggesting a gene-dosage effect. As in the ECE-1 (+/−) mice, the levels of APP and CTFβ were unchanged in the ECE-2 (−/−) mice compared with controls (Fig. 2).

**DISCUSSION**

The increase in Aβ concentration in the brains of ECE-deficient mice provides direct evidence of a physiological role for ECEs in limiting Aβ accumulation in the brain. It will be important in future studies to determine whether these elevations will enhance deposition and plaque formation either by themselves or when crossed with mouse models that normally deposit Aβ, such as the Tg2576 model (37). The same holds true for the neprilysin-efficient animals and for animals deficient in other enzymes that result in increased Aβ levels.

Given the evidence that Aβ plays a significant role in AD pathogenesis, factors that influence ECE activity, whether pharmacological, genetic, or other, may influence the risk for developing AD. The increases observed in the ECE-1 (+/−) animals are particularly noteworthy, as these animals have only a 27% reduction in ECE-1 activity (26), suggesting that even modest changes in ECE activity can have significant effects on Aβ accumulation. It will be interesting in future studies to determine whether variations in ECE activity correlate with either soluble and/or insoluble Aβ levels in human brain. Recent reports have suggested the linkage of a possible AD locus to chromosome 3, where two genes involved in Aβ catabolism, neprilysin and ECE-2, are located (38–40). Our results argue for the inclusion of ECE-2 in candidate gene approaches in this region.
Perhaps most immediately important is that ECE inhibitors have received a significant amount of pharmaceutical interest for their potential as drugs for the treatment of hypertension and other ailments (41–43). Our results indicate that if these drugs enter the brain they may increase APP levels, potentially leading to the development and/or acceleration of AD in susceptible individuals. Based on the genetic mutations that cause AD, in which APP levels are elevated for decades prior to the development of the disease, this potential side effect of ECE inhibitors may not be observed for years and should be considered carefully before clinical use of this class of compounds. Ideally, these drugs should be examined directly for any effects on Aβ in preclinical studies.

Finally, our data suggest a possible novel approach for reducing Aβ levels in vivo by up-regulating ECE activity. This could be accomplished, for example, through gene therapy, transcriptional activation, or perhaps even by reducing ECE inhibitors for years and should be considered carefully before clinical use of this class of compounds. Ideally, these drugs should be examined directly for any effects on Aβ in preclinical studies.

In summary, our data provide direct evidence that ECEs may degrade Aβ primarily intracellularly. Furthermore, Aβ degradation by ECEs may be involved in Aβ clearance. Thus, it appears that multiple enzymes are likely to be involved in Aβ catabolism in vivo. Catabolism of Aβ peptides by each of these enzymes would limit the accumulation of Aβ, and disruption of this catabolism may be a risk factor for AD. Because removal of Aβ by multiple enzymatic activities appears to contribute to overall Aβ catabolism, the identification of these activities should be viewed as complementary rather than mutually exclusive. For example, certain enzymes such as the ECEs may degrade Aβ predominately intracellularly. Following secretion, other enzymes such as NEP may play a larger role. In addition, the principle mechanism of Aβ removal may vary by cell type and brain region and could potentially be influenced by other factors such as inflammation. Understanding these processes in more depth may provide new clues to the abnormal accumulation of Aβ found in patients with Alzheimer’s disease.

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Aβ Levels Are Increased in ECE-deficient Mice

N. A., Price, D. L., Younkin, S. G., and Sisodia, S. S. (1996) Neuron 17, 1005–1013

33. Citron, M., Westaway, D., Xia, W., Carlson, G., Diehl, T., Levesque, G., Johnson-Wood, K., Lee, M., Seubert, P., Davis, A., Khodolenko, D., Matter, R., Sherrington, R., Perry, B., Yao, H., Strom, R., Lieberburg, I., Kimmens, J., Kim, S., Schenk, D., Fraser, P., St George Hyslop, P., and Selkoe, D. J. (1997) Nat. Med. 3, 67–72

34. De Jonghe, C., Cruts, M., Rogaeva, E. A., Tyroe, C., Singleton, A., Vanderstichele, H., Meschino, W., Derrant, B., Vanderhoeven, I., Backhovens, H., Vanmechelen, E., Morris, C. M., Hardy, J., Rubinsztein, D. C., St George-Hyslop, P. H., and Van Broeckhoven, C. (1999) Hum. Mol. Genet. 8, 1529–1540

35. Flood, D. G., Reaume, A. G., Dorfman, K. S., Lin, Y. G., Lang, D. M., Trusko, S. P., Savage, M. J., Annaert, W. G., De Strooper, B., Siman, R., and Scott, R. W. (2002) Neurobiol. Aging 23, 335–348

36. Nakano, Y., Kondoh, G., Kudo, T., Imairumi, K., Kato, M., Miyazaki, J. I., Tobiyama, M., Takeda, J., and Takeda, M. (1999) Eur. J. Neurosci. 11, 2577–2581

37. Hsiao, K., Chapman, P., Nilsson, S., Eckman, C., Harigaya, Y., Younkin, S., Yang, F., and Cole, G. (1996) Science 274, 99–102

38. Poduslo, S. E., Yin, X., Hargis, J., Brumback, R. A., Mastroianni, J. A., and Schwankhaus, S. (1999) Hum. Genet. 105, 32–37

39. Lorenzo, M. N., Khan, R. Y., Wang, Y., Tai, S. C., Chan, G. C., Cheung, A. H., and Marden, P. A. (2001) Biochim. Biophys. Acta 1522, 46–52

40. Tanzi, R. E., Kovacs, D. M., Kim, T. W., Moir, R. D., Guenette, S. Y., and Wasco, W. (1996) Neurobiol. Dis. 3, 159–168

41. Gray, G. A., and Webb, D. J. (1996) Pharmacol. Ther. 72, 109–148

42. Lofler, B. M. (2000) J. Cardiovasc. Pharmacol. 35, Suppl. 2, S79–S82

43. Kitas, E. A., Lofler, B. M., Daetwyler, S., Dehmow, H., and Aebl, J. D. (2002) Bioorg. Med. Chem. Lett. 12, 1727–1730

44. Telemaque, S., Emoto, N., deWit, D., and Yanagisawa, M. (1998) J. Cardiovasc. Pharmacol. 31, Suppl. 1, S548–S550

45. Hoang, M. V., and Turner, A. J. (1997) Biochem. J. 327, 23–26

46. Johnson, G. D., Stevenson, T., and Aha, K. (1999) J. Biol. Chem. 274, 4053–4058
