Catabolic attacks of membrane-bound angiotensin-converting enzyme on the N-terminal part of species-specific amyloid-β peptides

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ABSTRACT

Catabolic processes play a crucial role in the steady state of the amyloid-β peptide (Aβ). Neprilysin (NEP) and angiotensin-converting enzyme (ACE), two transmembranal enzymes with greatest importance in peptide pharmacology, are known to play a role in Aβ catabolism. This paper focuses on the N-terminal part of Aβ. This region contains the three amino acid residues that determine the differences between human (hAβ) and murine Aβ (mAβ). Moreover, the N-terminal part of Aβ contains the zinc-binding site of the molecule. Consequently, all hydrolytic attacks on this part of the Alzheimer peptide should be of exceptional interest. We investigated domain-selective forms of ACE in HPLC-monitored peptide degradation studies and used mass spectrometry for product analyses. We found that ACE-evoked a hydrolysis of the N-terminal part of m- and hAβ. The hAβ sequence hAβ (4–15) was found to be a better substrate for ACE compared to the corresponding murine form. Moreover, we localized the corresponding cleavage sites in the N-terminal part of Aβ as well as in the full-length molecule and identified new sites of endopeptidolytic attack by ACE. Finally, we demonstrate that both catalytic domains of mACE have similar hydrolytic activity on the N-terminal part of Aβ. Our results show that ACE besides its typical function as a dipeptidyl-carboxypeptidase has also unequivocal endopeptidolytic activities.

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1. Introduction

According to the amyloid-hypothesis, the central concentration of the amyloid-β peptide (Aβ) plays a crucial role in the development of Alzheimer's disease. The fragile steady state of extracellular Aβ is strongly influenced by two different peptidolytic processes — anabolic (for review see Hardy and Selkoe, 2002) and catabolic actions (review see Saido and Iwata, 2006; Wang et al., 2006). Over the last years, scientific interest focused on the catabolic activity of several peptidases on Aβ. NEP (neprilysin or neutral endopeptidase, EC 3.4.24.11), ACE (angiotensin-converting enzyme, EC 3.4.15.1), IDE (insulin-degrading enzyme, EC 3.4.24.56), ECE (endothelin-converting enzyme, EC 3.4.24.71) and plasmin (EC3.4.21.7) are involved in these processes (for review see Wang et al., 2006). Interestingly, most of these enzymes play also important roles in non-neuronal processes, and substances that interfere with the function of these enzymes are used as therapeutics. For example, ACE, a transmembranal dipeptidyl-carboxypeptidase, is involved in several angiotensin- or bradykinin-triggered renal, vascular, and cardiac processes. Thus, potent and specific inhibitors of ACE have been widely used in the treatment of cardiovascular disease and for other therapeutic purposes and belong to the top-selling drugs.

The amino acid sequences of Aβ peptides were strongly conserved in evolution. However, the N-terminal parts of the murine and the human Aβ molecule contain species-specific amino acid sequences. Three distinct differences in the N-terminal part (position 5 [Arg/Gly], position 10 [Tyr/Phe], and position 13 [His/Arg]) have been discussed to be responsible for the almost complete absence of Aβ aggregation and deposition in mice (Vaughan and Peters, 1981). Especially, the both mentioned membrane-bound peptidases ACE and NEP have been reported to cleave the Aβ molecule initially in its N-terminal section (for review see Wang et al., 2006). The NEP-derived Aβ degradation is well described (Howell et al., 1995; Iwata et al., 2000; Yasojima et al., 2001; Mohajeri et al., 2004). Notably, Iwata et al. (2000) found that NEP cleaves the Aβ molecule in position 9–10, in which structural differences exist between human and murine Aβ. Hu et al. (2001) reported that ACE degrades hAβ by cleaving between Asp^7-Ser^8. This attracts further attention, because it was the first observation of a unequivocal endopeptidolytic activity of the 'dipeptidyl-carboxypeptidase' ACE.

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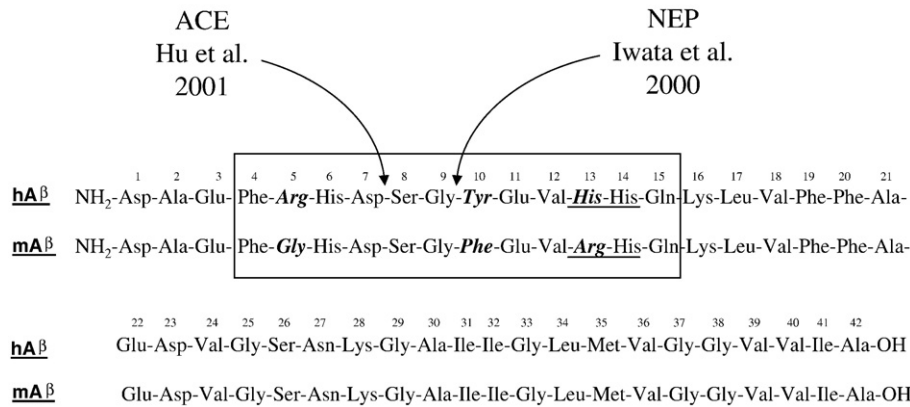E-mail address: siems@fmp-berlin.de (W.-E. Siems).

Fig. 1. Amino acid sequences of human (hAβ) and murine amyloid peptides (mAβ). Species-specific differences (human versus murine) are indicated in bold and Italics. Known N-terminal cleavage sites after NEP- (Iwata et al., 2000) and ACE-attacks (Hu et al., 2001) are indicated by arrows. Amino acids involved in Zn⁺⁺ binding sites are underlined. The N-terminal sequences hAβ (4–15) and mAβ (4–15) are framed.

In addition to these structural and catabolic relations, the N-terminal part of Aβ plays further important roles. Especially, the histidyl residues in position 13 and 14 in hAβ are found to be important for Zn^{2+}-binding and for the Zn-induced aggregation of the peptide (Liu et al., 1999; Miura et al., 2000; Kozin et al., 2001). The importance of the N-terminal part of Aβ and its degradation is also underlined by experiments using specific antibodies that reduce the plaque burden in Alzheimer's disease. Antibodies against the N-terminus of Aβ are very effective, while antibodies against the C-terminus of Aβ were not (Yang et al., 2000a,b; Chauhan and Siegel, 2005).

Considering that full-length Aβ (especially its human form) is difficult to handle, we focused on peptide sequence Aβ (4–15) that has adequate solubility and does not display spontaneous formation of oligomeric structures and associates. Aβ (4–15) already contains the interesting parts of the Aβ molecule: the three species-specific structural modifications, the Zn^{2+}-binding amino acids, and the described ACE- and NEP-cleavage sites.

The major aim of this study was to compare the action of ACE on the structurally different N-terminal parts of murine and human Aβ, and to describe respective cleavage sites. ACE has two nearly identical catalytic domains (Wei et al., 1992; Jaspard et al., 1993) that in spite of their homology, have different substrate properties (Rousseau et al., 1995; Deddish et al., 1998; Araujo et al., 2000). Consequently, these studies were also set up to clarify whether and how the both catalytic domains of ACE, the C- and the N-domain, are differently involved in the peptidolytic cleavage of Aβ.

2. Materials and methods

2.1. Materials

mAβ (1–40), hAβ (1–40), mAβ (1–42), hAβ (1–42) and the corresponding N-terminal partial sequences mAβ (4–15), hAβ (4–15), were
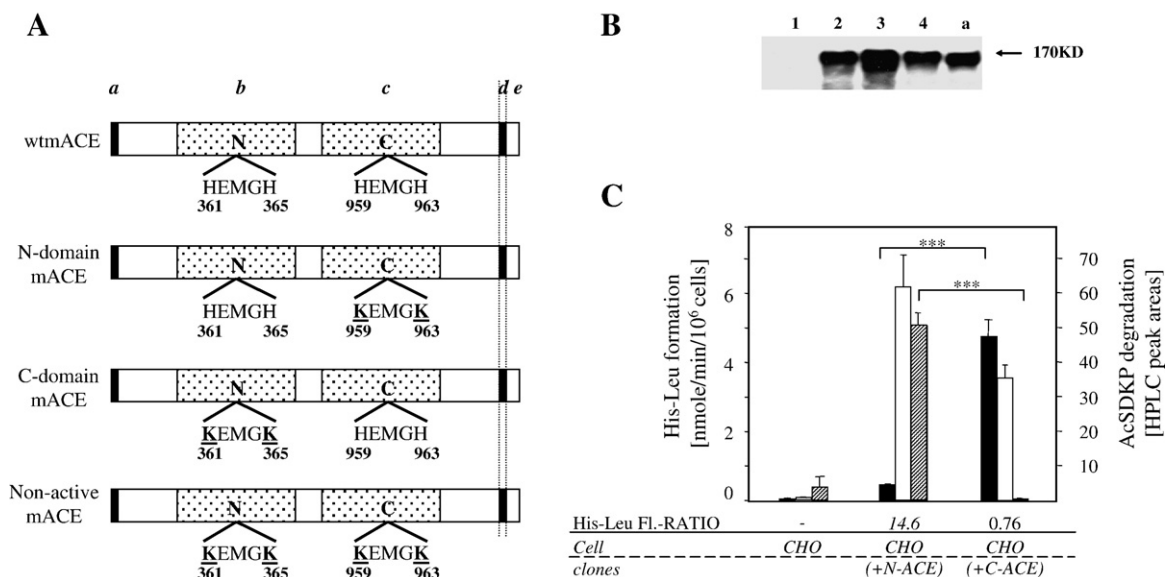

Fig. 2. Domain-selective forms of membrane-bound murine ACE (mACE), their molecular architecture, molecular mass and catalytic selectivity. (A) Schematic presentation of site-directed mutations in somatic murine ACE (mACE). Exchanged amino acids (His [H] versus Lys [K]) are underlined and in bold *Indicated ACE-segments*: (*a*) N-terminus; (*b*) catalytic N-domain; (*c*) catalytic C-domain; (*d*) transmembranal region; (*e*) C-terminus, cytoplasmatic tail; (*a–c*) extracellular parts. (B) Western blotting of the distinct forms of mACE expressed in CHO cells. (1) non-transfected CHO cells; (2) wtmACE-transfected CHO cells; (3) N-domain mACE-transfected CHO cells; (4) C-domain ACE-transfected CHO cells; as controls: (a) non-active ACE-transfected CHO cells. (C) ACE activities of selected domain-selective cell clones. CHO, non-transfected CHO cells; CHO (+N-ACE), N-domain mACE-transfected CHO cells; CHO (+C-ACE), C-domain mACE-transfected CHO cells. Used substrates (from left to right): Hip-His-Leu (black columns), Z-Phe-His-Leu (white columns), AcSDKP (hatched columns). Mean values with S.E.M., $n = 5$. The significance of differences for the degradation of domain-selective substrates (AcSDKP and Hip-His-Leu) by N- versus C-domain mACE-transfected cells was proved by t-tests. ***$p > 0.001$.