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ALTERATION OF PEPTIDES IN RAT BRAIN TREATED WITH ANGIOTENSIN-CONVERTING ENZYME INHIBITOR, CAPTOPRIL

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Abstract Although animal studies suggest that centrally active angiotensin-converting enzyme (ACE) inhibitors may protect against dementia beyond HTN control, the mechanism(s) underlying these improvements in cognitive function remains unclear. We measured the brain peptide levels in rat treated with captopril (50 mg/kg) for 3 weeks by using surface-enhanced laser desorption/ionization time-of-flight mass spectrometry (SELDI TOF-MS). Two protein chip arrays were used for peptide profiling: one with a strong anion- exchanger and the other with a weak cation-exchanger.

Comparing with control group, 15 mass peaks were considered specific to experimental animals, and 6 peaks were significantly up-regulated and 5 down-regulated.

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Key words: ACE inhibitors; captopril; memory

Introduction

Angiotensin-converting enzyme (ACE, EC3.4.15.1) plays a key role in the regulation of blood pressure through conversion of the inactive decapeptide angiotensin I to the vasoactive octapeptide angiotensin II as well as inactivation of the vasodilator peptide bradykinin[1]. ACE also has broad substrate specificity through its dipeptidyl carboxypeptidase and aminopeptidase activities toward various neuropeptides[1]. Inhibitors of ACE have been widely used in therapy against hypertension[2].

On the other hand, several reports[3,4] suggested a noble effect of ACE inhibitors that they improved the memory functions. ACE inhibitors that cross the blood-brain barrier (BBB), such as captopril, lisinopril, and trandrapril, are reported to be effective in preventing memory loss in hypertensive patients[5]. However, the mechanism of these effects is not clear, but important leads are emerging, because ACE may have the possible role in the regulation of neuropeptides, which concern the cognitive function, directly or indirectly.

Recently we have reported the function of rat brain ACE that may regulate Leu-Val-Val-hemorphin-7 (LVV-H7), sequence LVVYPWTORF for human, bovine and sheep: LVVYPWTORY for rat[6]. LVV-H7 was originally characterized as an opioid peptide that is produced from protease digestion of hemoglobin β-chain[7]. Furthermore, this decapeptide was isolated from sheep brain with nanomolar affinity to angiotensin IV receptor as a possible endogenous ligand for this receptor which is strongly correlated with memory function[8], and then reported as an insulin-regulated aminopeptidase[9]. Of interest, LVV-H7 was suggested to be associated with enhancement of memory function by ACE inhibitor treatment. ACE may hydrolyze some neuropeptides co-related with memory function, because ACE has broad substrate specificity. On the other hand, captopril may directly inhibit the metalloprotease which could play the key role of memory function.

In the present study, we measured the alteration of peptides in rat brain treated with captopril.

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Materials and Methods

Materials

Captopril was purchased from Sigma (St. Luis, Missouri, USA). Protein chip arrays were from Bio-Rad Lab. (CA, USA), and all other reagents were of analytical grade.

Animal treatment

Sprague-Dawley rats (male, 250-320 g) were treated with daily IP administration of captopril (50 mg/kg, n=4) for 3 weeks.

Extraction of brain peptides

Rat brains were homogenized with 9 vol. of 0.1%TFA, and then boiled in water-bath for 10 min. The cooled homogenates on ice were centrifuged at 25,000xg for 30 min at 4℃. The supernatants were applied on a column of C18 (2 x 5 cm), previously equilibrated with 0.1% TFA, and washed with 5 vol. of the same buffer. The peptides were eluted with 80%CH3CN/water containing 0.1% TFA. Elutes were evaporated to dryness, and removed TFA completely. The residue, which was resolved in water, was used for TOF-MS analysis.

SELDI-TOF MS analysis

Extracts were diluted 3 times by 20 mM acetate buffer, pH 5.0 and 50 mM Tris-HCl, pH 8.0, and then applied on two protein chip arrays (CM10: a weak cation-exchanger and Q10: a strong anion-exchanger). After washing out the excess of peptides and salts from chips, a-cyano-4-hydroxycinnamic acid as an energy-absorbing molecule was applied on. The chips were stand to dryness for over night at room temperature. Each chip was analyzed by SELDI-TOF-MS (Ciphergen Biosystems, Inc.).

Results and Discussion

Profiling of rat brain extracts with CM10

Only one mass peak (m/z = 657) was detected in control groups (n=4) and these peaks disappeared in captopril treated groups (n=4) at the condition of pH 5 (Fig. 1). More five mass peaks were detected in control at the condition of pH 8 (Fig. 2). These peaks disappeared in captopril treated groups as well as above condition.

In captopril groups, two mass peaks (m/z = 4508 and 2528), which could not detect in control groups, appeared in both pH 5 and 8 (Fig. 1 and 2).

Profiling of rat brain extracts with Q10

At the condition of pH 5, fore mass peaks (m/z = 1982, 2095, 2925, 3072) were detected in captopril treated groups, and one mass peak was detected in control groups (Fig. 3). A weak mass peak (m/z = 4513) was detected in captopril treated groups at the condition of pH 8 (Fig. 4).

In this peptide profiling in rat brain, it was shown that the expression of small peptides in control groups were suppressed by the treatment with captopril (Fig. 1-3). It is well known that captopril can bind Zn in the protein. It suggests that captopril may inhibit the some metalloproteases, especially zinc metalloproteases including ACE, which could contribute the production of these peptides from precursors. Further structural analyses of these peptides are required to clarify their relations to ACE inhibition and roles in central nervous system function, particularly in memory.

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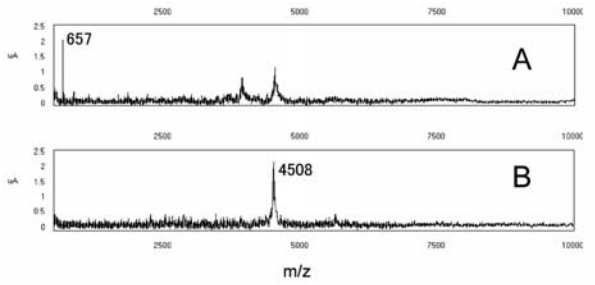

Figure 1 Profiling of rat brain extracts with CM10 at pH 5, A: control, B: captopril treated.

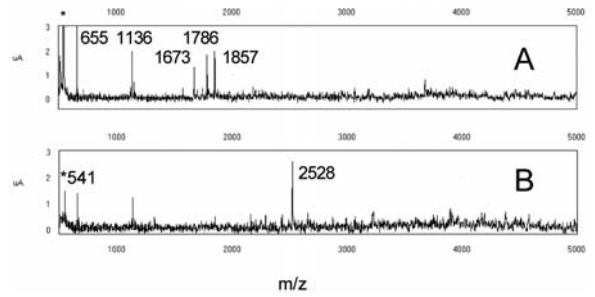

Figure 2 Profiling of rat brain extracts with CM10 at pH 8, A: control, B: captopril treated.

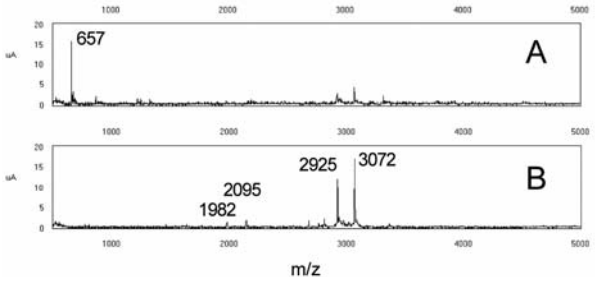

Figure 3 Profiling of rat brain extracts with Q10 at pH 5, A: control, B: captopril treated.

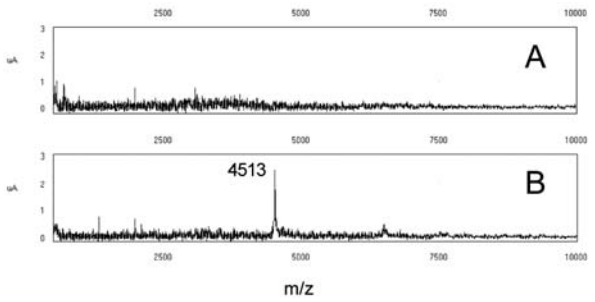

Figure 4 Profiling of rat brain extracts with Q10 at pH 8, A: control, B: captopril treated.