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INHIBITION OF ANGIOTENSIN CONVERTING ENZYME OF RAT BRAIN WITH BRADYKININ AND ITS FRAGMENTS

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SUMMARY

Bradykinin and its fragments, such as Arg-Pro, Arg-Pro-Pro and Phe-Ser-Pro, competitively inhibited the activity of angiotensin converting enzyme (dipeptidyl carboxypeptidase; E.C. 3.4.15.1) in the microsomal fraction of rat brain, and their Ki values were 0.76, 16, 5.6 and 5.2 μM, respectively, using hippuryl-L-histidyl-L-leucine as the substrate. These results suggest that bradykinin and its fragments compete with angiotensin I for the active site of the brains converting enzyme.

INTRODUCTION

The presence of the renin-angiotensin system in the brain has been demonstrated by many investigators (1,2,3). Of this system, the enzyme converting angiotensin I to angiotensin II by removing the C-terminal histidyl-leucine (dipeptidyl carboxypeptidase; E.C. 3.4.15.1) was shown to be present at a higher concentration in the striatum and the cerebellum of rats (2), and in the caudate nucleus of humans (4). In the lung, kidneys and plasma, the enzyme was established to be identical with kininase II which cleaves the C-terminal phenylalanyl-arginine from kinins (5,6,7). However, it has been unclear whether the angiotensin converting enzyme in the brain has the ability to cleave the C-terminal dipeptide from kinins.

The activity of the converting enzyme in the lung is potently inhibited by bradykinin in vitro and in situ, and this inhibition mechanism has been suggested to be due to its competition with angiotensin I for the active site of the enzyme (5,8,9). The above evidence has led to elucidating the connection

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between the renin-angiotensin system and kinin in the brain. This paper details the influence of bradykinin and its fragments on the activity of angiotensin converting enzyme of the rat brain.

MATERIALS AND METHODS

Male Sprague-Dawley rats, weighing 250 - 350g, were anesthetized with pentobarbital Na (30 mg/kg, i.p.). Both carotid arteries were cannulated and cold saline was infused for removing blood from the brain. After exsanguinating from the jugular veins, the whole brain was removed and homogenized with 9 vol. of 0.32 M sucrose containing 10 mM Tris-HCl buffer (pH 7.0). Subcellular fractions were prepared by differential centrifugation according to the method described by Whittaker and Barker (10). All pellets were suspended in the appropriate volume of 50 mM Tris-HCl buffer, pH 7.4, and the final supernatant was dialyzed against 100 vol. of the buffer overnight.

The activity of the enzyme was assayed fluorometrically using hippuryl-His-Leu (HHL) as a substrate according to the procedure of Yang and Neff (2), and using angiotensin I following the procedure of Tsai and Peach (11).

Kininase activity was determined by bioassaying residual bradykinin with the isolated guinea pig ileum, after the incubation of an enzyme preparation in 50 mM Tris-HCl buffer (pH 7.4) containing 2 μM bradykinin and 0.1 M NaCl.

Protein determination was made by the micro-biuret method of Itzhaki and Gill (12).

The peptide fragments derived from bradykinin were synthesized in our laboratory by liquid phase synthesis (13).

RESULTS

As shown in table 1, the highest specific activity of converting enzyme for HHL was located in the microsomal fraction. The results agreed with previous reports (2,14). Therefore, the subsequent experiments were carried out using the microsomal fraction as the enzyme preparation. Kininase activity was exclusively distributed in the soluble supernatant, which was in disagreement with the distribution of the converting enzyme.

Fig. 1 shows the dose-dependent inhibition of the converting enzyme activity of bradykinin. The concentration of bradykinin required for 50 % inhibition was 1.9×10^{-6} M for HHL or 2.0×10^{-5} M for angiotensin I. The plots of the effect of bradykinin on enzyme activity at different substrate concentrations are included in Fig. 2. Km values calculated from the data were 9.1×10^{-4} M for HHL and 3.7×10^{-5} M for angiotensin I. Bradykinin acted as a competitive inhibitor for the enzyme activities in both substrates. The Ki of bradykinin was calculated to be 7.6×10^{-7} M for HHL and 1.3×10^{-5} M for

Table 1. Subcellular distributions of the specific activity of angiotensin converting enzyme and kininase in the rat brain

Fraction	Specific activity (nmole/mg protein/min)	
	Angiotensin converting enzyme	Kininase
Whole homogenate (3)	0.31 ± 0.01	1.15 ± 0.08
Nuclei & unbroken cells (4)	0.12 ± 0.06	0.22 ± 0.02
Crude mitochondria (4)	0.33 ± 0.06	0.74 ± 0.10
Microsome (4)	0.63 ± 0.25	0.55 ± 0.06
Supernatant (4)	0.06 ± 0.02	5.65 ± 0.58

Values represent means ± S.D.. Number of experiments are shown in parentheses. Angiotensin converting enzyme activity was assayed using HHL as the substrate.

angiotensin I, using a standard equation for estimating the Ki of competitive inhibitors.

Some peptide fragments, such as Arg-Pro, Arg-Pro-Pro and Phe-Ser-Pro, had inhibiting effects on the enzyme, and their Ki values were 1.6×10^{-5}, 5.9×10^{-6} and 5.2×10^{-6} M respectively as shown in Fig. 3. But, Pro-Pro, Pro-Pro-Gly, Pro-Pro-Gly-Phe, Pro-Gly, Pro-Gly-Phe, Gly-Phe-Ser, Gly-Phe-Ser-Pro, Ser-Pro, Ser-Pro-Phe-Arg and Phe-Arg showed no effect at a concentration of 2.5×10^{-5} M for HHL and 2.5×10^{-4} M for angiotensin I.

DISCUSSION

Present experiments showed that angiotensin converting enzyme in the microsomal fraction of the rat brain was inhibited by bradykinin competitively. This result agrees with the previous reports on the enzyme found in rabbit lungs in vitro (8,9). Some peptide fragments derived from bradykinin, especially N-terminal di- and tripeptide, Arg-Pro and Arg-Pro-Pro, and Phe-Ser-Pro, also had an inhibitory effect by a competitive mechanism. The tripeptide Arg-Pro-Pro was reported to be a competitive inhibitor of the enzyme purified from hog lungs using hippuryl-Gly-Gly as a substrate and was given the Ki value of 6.0×10^{-6} M (15). The Ki of the tripeptide coincides with that in our present experiment using the microsomal fraction of rat brain for the

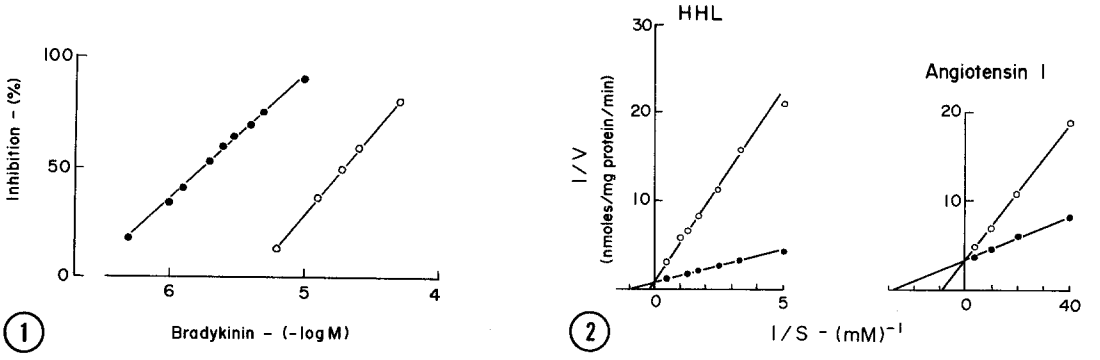

Fig. 1. Dose-dependent inhibition of brain angiotensin converting enzyme by bradykinin. -●- HHL as the substrate, -o- Angiotensin I as the substrate. Points are the mean values of 3 experiments.

Fig. 2. Double reciprocal plots of the activity of angiotensin converting enzyme of the rat brain in the presence of bradykinin. Left, HHL as the substrate in the presence or absence of bradykinin (5.0×10^{-6} M); Right, Angiotensin I as the substrate in the presence or absence of bradykinin (2.5×10^{-5} M). -●- No addition, -o- Bradykinin.

enzyme preparation. These results support the hypothesis that bradykinin and some of its fragments do compete with angiotensin I or a synthetic substrate for the active site of the enzyme found in the brain as well as in the lung. Benuck and Marks (16) have reported that Bothrops jararaca nona peptide (SQ 20881) and its analog (SQ 14225) also competitively inhibit the angiotensin converting enzyme purified from rat brains, or the lung. Although this data suggests a strong similarity between the properties of the converting enzyme of the lung and brain, it has not been confirmed that the enzyme in the brain has the kininase activity.

Among the peptide fragments used, Arg-Pro, Arg-Pro-Pro and Phe-Ser-Pro had more potent inhibitory effect on the enzyme activity than any other peptides examined. Comparing structure of various B. jararaca peptides, which inhibit converting enzyme indicates that the C-terminal proline group is essential (17). Furthermore, it emphasized the importance of the last three amino acid residues at the C-terminus of inhibitors for binding to the active site of the enzyme (17). Cushman et al. showed that active analogs of BPF_{5a} (⍺Glu-Lys-Trp-Ala-Pro), the most potent inhibitor of snake venom, had the

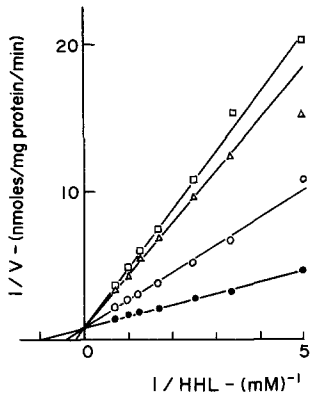

Fig. 3. Double reciprocal plots of angiotensin converting enzyme in the presence of peptides. HHL was used as the substrate. -●- No addition, -o- Arg-Pro (2.5×10^{-5} M), -Δ- Arg-Pro-Pro (2.5×10^{-5} M), -□- Phe-Ser-Pro (2.5×10^{-5} M).

C-terminal tripeptide sequence Phe-Ala-Pro and the tripeptide itself was active in enzyme inhibition (17). In this respect, it is interesting to note the structural resemblance between Phe-Ser-Pro and the C-terminus of BPF_{5a}, even though the penultimate residues differ.

In the degradation of bradykinin, angiotensin converting enzyme may play an important role in the brain, just as in the peripheral tissues. Moreover, in addition to bradykinin, its degradation products such as Arg-Pro-Pro and Phe-Ser-Pro might block the conversion of angiotensin I in the brain.

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