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ATRIOPEPTIN 2 IS HYDROLYSED BY CARDIAC BUT NOT PULMONARY ISOZYME OF ANGIOTENSIN-CONVERTING ENZYME

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Hydrolysis of Bz-Gly-Ser-Phe-Arg , C-terminal fragment of atriopeptin 2, by human cardiac angiotensin-converting enzyme has been studied. The K_M for the reaction was 10^{-4} M. The effect of concentration of NaCl on activity of cardiac angiotensin-converting enzyme has been determined, which allowed to regard Bz-Gly-Ser-Phe-Arg as bradykinin-like substrates. It was demonstrated that cardiac, but not pulmonary isozyme of angiotentin-converting enzyme specifically hydrolyses atriopeptin 2.

Angiotensin-converting enzyme (ACE, EC 3.4.15.1.), a metallopeptidase, was found in various human and animal tissues and fluids(1,2). The enzyme is most abundant in lung, testes, and kidney (1,2). At present, isozymes isolated from lung, kidney, heart, testes, liver, and other sources have been characterized (3-6). The enzyme isolated from various organs differ in immunological, molecular, and catalytic properties. These data testifying to the organ specifity of angiotensin-converting enzyme (1,5,7). ACE has a broad substrate specifity and hydrolyses angiotensin 1, bradykinin, enkephalins and their analogs, luteinizing hormone-releasing hormone, substance P, and other active peptides, cleaving C-terminal di- or tripeptide (8-10). It has been demonstrated that dipeptidylcarboxyhydroxylase isolated from bovine atrium cleaves C-terminal fragment from atriopeptin 2

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(11), one of atrium natriuretic peptides modulating natriuresis and diuresis (12). We suggested that the formation of atriopeptin 1 from atriopeptin 2 occurs in heart, and ACE is involved in this process. Here we report hydrolytic cleavage of atriopeptin 2 and its C-terminal analog by ACE isolated from human lung and heart.

METHODS

Angiotensin-converting enzyme was isolated from human lung and heart as described (4). Atriopeptin 2 (Ser-Ser-Cis-Phe-Gly-Gly-Arg-Ile-Asp-Arg-Ile-Gly-Ala-Gly-Ser-Gly-Leu-Gly-Cis-Asp-Ser-Phen-Arg) and its C-terminal analog (Bz-Gly-Ser-Phe-Arg) were synthetized as described (13).

The degradation of atriopeptin 2 was studied in 0.01 M phosphate buffer (pH 7.4) containing 0.2 M NaCl for 6 h at 37°C in the presence or absence of captopril. In inhibition experiments enzyme preparations were preincubated with the inhibitor for 30 min at 37°C. Spontaneous hydrolysis of atriopeptin 2 was taken into account in each experiment. The reaction was stopped by adding trifluoroacetic acid (final concentration 15% w/v). Aliquots were analysed using HPLC on a Spherisorb ODS-column (4.6 x 250 mm) equilibrated with 0.05 M KH_2PO_4 (pH 3.0) containing 10% acetonitrile. The products were eluted with a 25 min acetonitrile gradient (10-60%) and detected by measuring optical density of the eluate at 214 nm. The degree of hydrolysis was calculated from the decrease of the area of the peak emerging 17 min after the beginning of elution.

Enzyme hydrolysis of Bz-Gly-Ser-Phe-Arg was usually carried out in 0.01 M phosphate buffer (pH 7.4) containing 0.15 M NaCl and substrate (10^{-3} M) at 37°C. Concentration of cardiac ACE was 10^{-9} M. Aliquots taken at various periods were analysed with HPLC on a Spherisorb ODS-column (4.6 x 250 mm) equilibrated with 0.05 M KH_2PO_4 (pH 3.0) containing 10% acetonitrile. The products were eluted with a 20 min acetonitrile gradient (10-60%) and detected as in previous experiment. The degree of the hydrolysis was calculated from the decrease of the area of the peak emerging 16 min after the begining of elution.

RESULTS AND DISCUSSION

The hydrolysis of Bz-Gly-Ser-Phe-Arg in the presence of human cardiac ACE was determined using HPLC (Fig. 1). To optimize the process the effect of NaCl concentration on the activity of ACE was examined. As seen from Fig. 2, at elevated concentrations of NaCl up to 0.7 M the efficiency of catalysis remains unchanged. Higher concentrations of NaCl reduced the enzyme activity. This suggests that the peptide can be referred to as bradykinin-like substrates, because this group of substances is cleaved by ACE

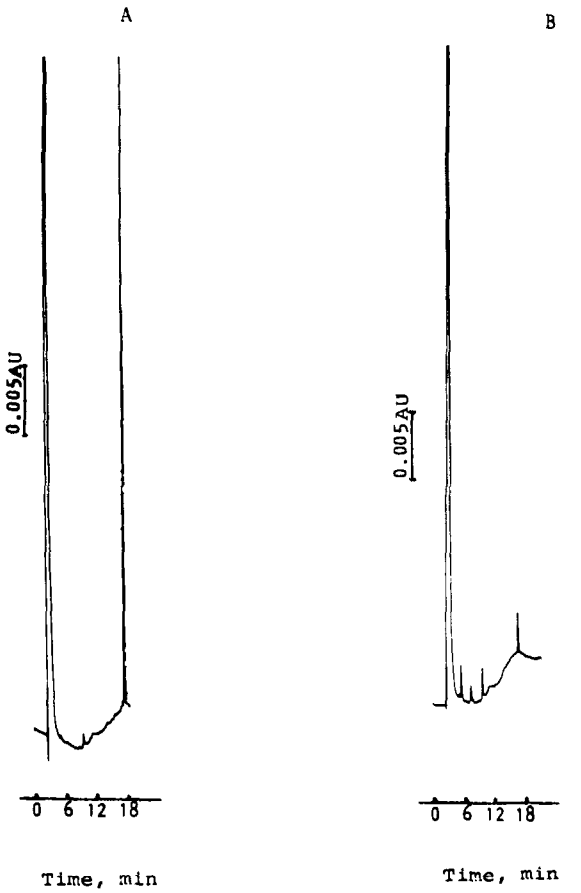

Fig. 1. Hydrolysis of Bz-Gly-Ser-Phe-Arg by human cardiac angiotensinconverting enzyme. Experimental conditions are described under "Methods". Time of hydrolysis was (A). 0 min; (B). 15 min.

with constant velocity independent of Cl^- concentration (14). This phenomenon is reasonable , because atriopeptin 2 and bradykinin have the same C-terminal dipeptide (Phe-Arg).

Michaelis constant for cardiac ACE (10^{-4} M) was determined by varying the initial concentration of Bz-Gly-Ser-Phe-Arg. This value coincides with K_M for bovine atrium dipeptidylcarboxyhydroxylase obtained for the same substrate (11). It is noteworthy that human heart and bovine atrium peptidases have considerable similarities though the activation of these enzymes with monoanions is different (4,11). It should be mentioned that human cardiac ACE is not inhibited by the Bz-Gly-Ser-Phe-Arg, which is

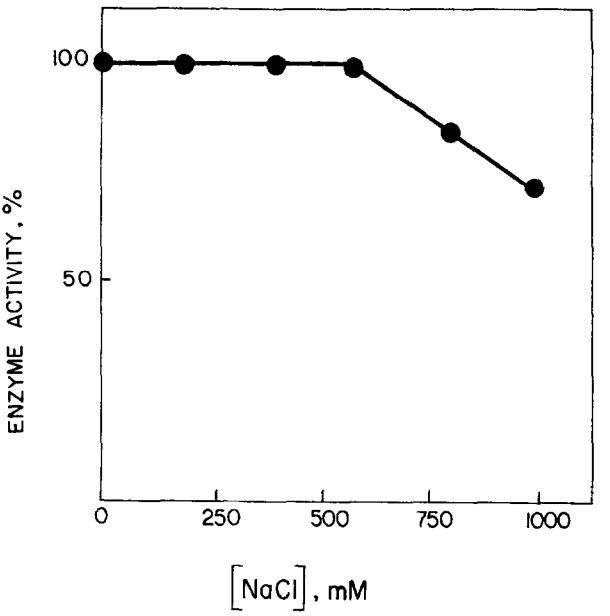

Fig. 2. Effect of NaCl concentration on the activity of cardiac angiotensin-converting enzyme.

characteristic of Bz-Gly-His-Leu (4), C-terminal analog of angiotensin 1. Bovine atrium dipeptidyl carboxyhydrolase also is not inhibited by C-terminal analog of atriopeptin 2 (11).

It was shown that pulmonary ACE produces no structural changes in a molecule of atriopeptine 2 (Table 1), which agrees with the data reported by Lanzillo (15). Unlike pulmonary isozyme, cardiac ACE effectively hydrolysed atriopeptin 2 and the degree of the peptide cleavage depends on the enzyme concentration (Table 1). The peptide hydrolysis was completely suppressed by captopril, specific inhibitor of ACE.

Table 1. Hydrolysis of atriopeptin 2 by human isozymes of angiotensin-converting enzyme

enzyme concentration (M)	source of enzyme	concentration of captopril (M)	degree of atriopeptin 2 hydrolysis (%)
1×10^{-9}	heart	-	0
3×10^{-9}	heart	-	10
3×10^{-8}	heart	1×10^{-6}	0
1×10^{-8}	heart	-	55
1×10^{-8}	heart	1×10^{-6}	0
1×10^{-8}	lung	-	0

Thus, this study provides direct evidence for specific hydrolysis of both atriopeptin 2 and its C-terminal fragment by human cardiac ACE, but not by pulmonary ACE. This data confirms the assumption (11) that atriopeptin 1 is formed in myocardium and ACE is involved in this process. In addition, the functional differences between cardiac and pulmonary isozymes in respect to atriopeptin 2 is another experimental support for organ-specifity of angiotensin-converting enzyme.

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