INHIBITION BY SUBSTANCE P OF ACTIVITY OF AN ANGIOTENSIN-CONVERTING ENZYME IN HUMAN BLOOD SERUM

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It has been shown [14] that the activity of angiotensin-converting enzyme (ACE) from the rat corpus striatum is inhibited by the neurotransmitter known as substance P (SP). This work may be of great importance to the elucidation of interaction between different polypeptide mediator systems of nerve tissue. The role of ACE in the regulation of arterial pressure, and also information obtained on the effect of SP on renin secretion [8] and the tone [17, 19] and permeability [9] of microvessels served as the basis for the present investigation. ACE from human blood, with respect to its chief physicochemical characteristics, is identical with the enzyme obtained from other sources [12].

In the investigation described below the effect of synthetic SP and its structural derivatives was studied on ACE activity in human blood serum.

EXPERIMENTAL METHOD

Blood serum from healthy donors was used. Samples of serum, divided into small portions, were kept at −20°C. ACE activity was determined by the method in [7] in our own micromodification, which allowed much smaller quantities of substrate to be used. Hippuryl-histidyl-leucine was used as the the substrate. The histidyl-leucine formed under the influence of ACE gives a fluorogen with o-phthalic dialdehyde in an alkaline medium, the quantity of which was determined in a Hitachi (Japan) spectrofluorometer at λ_{ex} = 360 nm and λ_{em} = 500 nm. The following reagents were used: hippuryl-histidyl-leucine and histidyl-leucine were synthesized by A. G. Terent'ev (Institute of Molecular Biology and Genetics, Academy of Sciences of the Ukrainian SSR, Kiev) and by Henklein (Drug Research Institute, East German Academy of Sciences, Berlin); the SP and its derivatives Arg-Pro-Lys-Pro-3CH_3COOH and Gln-Gln-Phe-Phe-Gly-Leu-Met-NH_2 were synthesized by Bienert, from the same institute in Berlin, Lys-Pro-2HBr and Z-Arg-(NO_2)-Pro were obtained from Neubert, Martin Luther University, Halle, East Germany; the o-phthalic dialdehyde was from Koch-Light, England; the captopril was obtained from Squibb, USA.

EXPERIMENTAL RESULTS

The initial ACE activity from the blood serum was 27.2 ± 1.3 nmoles His-Leu/ml·min (n = 11), in agreement with data in the literature [7]. Captopril inhibited ACE activity over a wide range of concentrations (Fig. 1). The value of I_{50} (inhibition constant) for this inhibitor was found to be similar to that based on results obtained by other workers [5, 20]. SP inhibited ACE within a concentration range from 10 to 100 μM; I_{50} = 31 μM. This value is comparable with that for inhibition by SP of ACE from rat brain [14].

To determine the functional role of the individual parts of the SP molecule experiments were carried out with different fragments of this polypeptide. The greatest inhibitory activity was linked with the Arg-Pro-Lys-Pro (SP_{1-4}) and Lys-Pro (SP_{3-4}) fragments (Table 1; Fig. 2). The dipeptide Arg-Pro (SP_{1-2}) and the C-terminal part of the SP_{5-11} molecule inhibited ACE activity weakly.

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TABLE 1. I_{50} of SP and Its Derivatives for Inhibition of ACE from Human Blood Serum

Substance	Structure	I_{50}, μM
SP	1 2 3 4 5 6 7 8 9 10 11 Arg-Pro-Lys-Pro-Gln-Gln-Phe-Phe-Gly-Leu-Met·NH_2	31
SP_{1-11}		
SP_{1-2}	Arg-Pro	>200
SP_{3-4}	Lys-Pro	20
SP_{1-4}	Arg-Pro-Lys-Pro	29
SP_{5-11}	Gln-Gln-Phe-Phe-Gly-Leu-Met·NH_2	>200

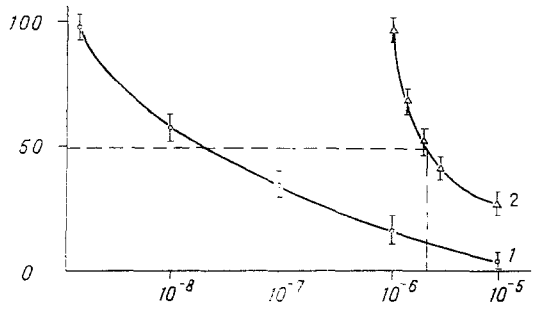

Fig. 1. Inhibition of ACE activity from human blood serum by captopril (1) and SP (2). Abscissa, concentration of inhibitor (M); ordinate, residual ACE activity (in %). 1) Captopril, 2) SP.

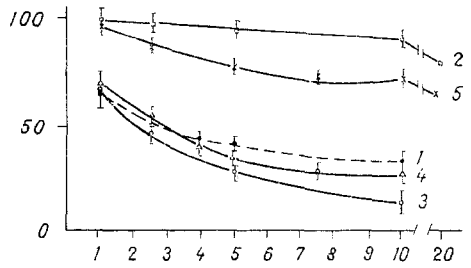

Fig. 2. Inhibition of ACE activity by SP and its structural derivatives. 1) SP (SP_{1-11}), 2) SP_{1-2}, 3) SP_{3-4}, 4) SP_{1-4}, 5) SP_{8-11}. Abscissa, concentration of inhibitor ($\times 10^{-5}$ M); ordinate, residual ACE activity (in %). Amino-acid composition of polypeptides given in Table 1.

Cushman et al. [5, 6] built a hypothetical model of the active center of ACE, where the functional groups and binding sites with the inhibitor were determined. The validity of this hypothesis was confirmed by synthesis of highly effective inhibitors of ACE, being derivatives of L-proline. The presence of an amino-acid residue of proline in the SP molecule as a condition for blocking of the active center of ACE was confirmed by the present experiments also: SP_{1-4} and SP_{3-4} proved to be effective inhibitors of the enzyme. However, according to Cushman's hypothesis, the C-terminal proline ought to have a free carboxyl group and, consequently, not the whole molecule, but the hydrolyzed molecule of the polypeptide, participates in the inhibition of ACE by SP. Evidence in support of this view is given by the similar values of I_{50} for SP_{1-11}, SP_{1-4}, and SP_{3-4} (Table 1). In principle, hydrolysis of the SP molecule in the present experiments could take place on account of dipeptidyl-peptidase IV (EC 3.4.14.1) [11, 12], introduced into the incubation medium with the blood serum. It is also an interesting fact that the dipeptide SP_{1-2}, in which proline is bound directly to arginine, exhibits weak ACE-inhibiting activity. Although the arginine and lysine molecules are very similar in their structure, the Lys-Pro type of bond is more effective for blocking the functional groups of the enzyme.

Inhibition of ACE *in vivo* leads to a decrease in formation of the pressor peptide angiotensin II, preservation of the bradykinin level and, correspondingly, a fall in the arterial blood pressure. However, the values we obtained for I_{50} for SP were several orders of magnitude higher than the quantities of this substance found in the blood [4, 18] or effective doses of the polypeptide by intravenous injection [3, 15, 16].

Besides views on the general physiological hormone-modulating role of the polypeptide circulating in the blood [1], data have also been obtained to show that SP participates in the local regulation of the microcirculation and, in particular, in degranulation of mesenteric mast cells [2], reactions of inflammation and antidromic vasodilatation of the skin vessels [13], etc. In the case when regulation of the microhemodynamics is coupled with local, but well-marked changes in concentration of the mediator, it can be postulated that SP has a regulating effect on the activity of tissue or plasma ACE.

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EFFECT OF INOTROPIC FACTORS ON POSTEXERCISE CHARACTERISTICS OF THE HEART

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The pumping function of the heart, like that of any pump, is largely determined by its ability to overcome the load at the output and, as has been recently shown [2, 3] it can be described by means of postexercise or output characteristics, i.e., the relationship between intraventricular pressure and blood flow in the aorta during constant filling. There have been few investigations into the study of factors influencing postexercise curves. It has been shown that paired stimulation of the isolated cat's heart with a hydraulic model of the aorta [4] causes a rotation shift of the postexercise curve around the extrapolated value of the blood flow at zero pressure, thereby increasing the extrapolated value of the pressure at zero blood flow. Isoproterenol, as has been calculated on a model of the left ventricle (using the method of Fourier analysis of pressure and blood flow curves), increases whereas propanol reduces the extrapolated value of the blood flow at zero load [5].

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