BIOPHYSICS AND BIOCHEMISTRY

Biochemical and Physiological Activity of New Peptide Inhibitors of Angiotensin Converting Enzyme

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 122, No. 9, pp. 279-281, September, 1996
Original article submitted March 6, 1996

The physiological and biochemical activity of new angiotensin converting enzyme inhibitors is studied *in vitro* (in microsomal fractions from the pituitary gland and corpus striatum) and *in vivo*. Compound PP-09, an N-carboxyalkyl derivative of enalapril, displaying high inhibiting activity towards rat serum and tissue angiotensin converting enzyme and lowering arterial pressure in spontaneously hypertensive rats, is selected.

Key Words: *angiotensin converting enzyme; inhibitors; spontaneously hypertensive rats*

Angiotensin converting enzyme (dipeptidyl carboxypeptidase, EC 3.4.15.1, ACE) is a key regulatory factor of the renin-angiotensin and kinin systems. The extensive search for new ACE inhibitors and their wide application in clinical practice, primarily in cardiology, are dictated by the biochemical and pathophysiological significance of ACE [1,2,5,12]. The ACE inhibitors (captopril, methiopril, ramipril, enalapril, etc.) reduce arterial pressure (AP) and prevent myocardial hypertrophy and postischemic arrhythmias [3,6,8,9]. The search for new ACE inhibitors is based on the concept of tissue-specific localization of ACE [4,7].

In the present study we assessed the biochemical and physiological activity of some new ACE inhibitors, enalapril N-carboxyalkyl derivatives, synthesized at the Institute of Biomedical Chemistry (Russian Academy of Medical Sciences).

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MATERIALS AND METHODS

Synthesis of preparations. Original compounds PP-05, PP-06, PP-07, PP-08, and PP-09, the derivatives of α-N-alkylated dipeptide, were synthesized by Dr. V. F. Pozdnev. For the synthesis of 1-carboxy-2-(benzylaminocarbonyl)ethyl derivatives potassium salts of the dipeptides Trp-Pro-OH, Ala-Pro-OH, and Met-Pro-OH (all amino acids are L-isomers) were alkylated with N-benzylmaleimide, which yields cyclic conjugates in the form of a diastereoisomer mixture (PP-05, PP-06, and PP-07, respectively). These products were hydrolyzed with alkali and neutralized, after which compounds PP-08 (1-carboxy-2-(benzylaminocarbonyl)ethyl-tryptophanyl-proline) and PP-09 (1-carboxy-2-(benzylaminocarbonyl)ethyl-alanyl-proline) were obtained in the form of chromatographically homogenous crystalline powders.

ACE activity assay. Angiotensin converting activity was determined spectrofluorimetrically using Z-Phe-His-Leu (ZFHL) as a substrate [11]. A substrate-buffer solution containing 10 μl 25 mM ZFHL

0007-4888/96/0009-0895$15.00

TABLE 1. Effect of Compound PP-05—PP-09 on ACE Activity in Various Rat Organs *In Vitro* (IC_{50}, μM; substrate — ZFHL)

Tissue	Inhibitors					
	PP-05	PP-06	PP-07	PP-08	PP-09	captopril
Serum	7.5	2.5	50	3.0	0.001	0.01
Heart	8.0	5.0	70	0.07	0.005	0.01
Pituitary	4.0	2.0	18	2.5	0.025	0.01
Striatum	4.0	4.0	20	1.0	0.005	0.01

Note. n=6-8 for each experimental series.

and 200 μl 0.1 M potassium-phosphate buffer, pH 8.3, was incubated with enzyme sample (40 μl) for 30 min at 37°C. The reaction was stopped with 0.28 N NaOH. Then o-phthaldialdehyde was added, and the reaction was stopped with 3 N HCl. Fluorescence was measured at E_{ex}=360 nm and E_{em}=500 nm. Compounds PP-05—PP-09 were preincubated with the enzyme for 10 min prior to the addition of substrate.

Animal experiments. Ex vivo studies were carried out on Wistar male rats weighing 180-200 g. Compound PP-09 was dissolved in methanol, adjusted to working concentration with 0.9% NaCl, and injected intraperitoneally in a dose of 20 mg/kg. The animals were decapitated after 1 and 4 h, and ACE activity was assayed in serum and in homogenates of striatum and pituitary gland.

Special series of experiments was performed on spontaneously hypertensive rats (SHR, males weighing about 300 g). Wistar-Kyoto rats of the same age and weight served as controls. The animals were maintained under standard vivarium conditions. Systemic AP and heart rate were measured using a PE-50 polyethylene catheter (0.58 mm internal diameter) implanted under Nembutal anesthesia (40 mg/kg) into the carotid artery. The ends of the catheter were fixed in the scapular region. On the day of experiment an alert rat was placed in a cage, where it could move freely. The catheter was connected with a Statham pressure transducer, and AP was recorded throughout the experiment.

Reagents. ZFHL and o-phthaldialdehyde were purchased from Sigma, sucrose, Tris, and NaH_2PO_4 from Serva, other reagents (chemically pure grade) were of domestic manufacturing. All solutions were prepared using deionized water.

RESULTS

Compounds PP-05, PP-06, PP-07, PP-08, and PP-09 were tested *in vitro* on microsomal fractions of ACE obtained from various rat tissues. Compound PP-07 exhibited the minimal activity. Compound PP-09 showed the maximum activity: its inhibition constant was comparable to that of captopril (Table 1). A certain tissue selectivity was noted for PP-08 with maximum inhibiting activity towards ACE from the heart microsomal fraction. Inhibiting activity of PP-09 was different in different tissues, being maximal in the serum, heart, and striatum (the lowest IC_{50} values). Since PP-09 exhibited the maximum inhibiting activity, this compound was used in subsequent experiments.

Table 2 shows the effect of intraperitoneal injection of PP-09 on ACE activity in rat serum, pituitary gland, and striatum. PP-09 markedly inhibited ACE activity (to 50%) in these tissues 1 h postinjection, whereas in animals sacrificed 4 hours postinjection ACE activity did not differ from the control; moreover, serum ACE activity was 27% higher than in the control.

TABLE 2. Effect of Compound PP-09 of ACE Activity *Ex Vivo* ($M \pm m$, n=5)

Tissue	Time after injection of PP-09			
	1 h		4 h	
	control	experiment	control	experiment
Serum	1.89±0.26	0.93±0.09* (-51)	1.66±0.22	2.12±0.15 (+27)
Pituitary	20.56±1.69	11.14±1.83* (-46)	18.48±2.23	16.89±1.97
Striatum	14.72±1.48	8.23±1.75* (-45)	14.63±1.36	14.79±3.08

Note. ACE activity is expressed His-Leu, nmol/min×mg protein. Substrate — ZFHL. *p<0.05 compared with the control. Percent of differences between experimental and control samples is given in parentheses.

The next series of experiments was carried out on rats with genetically-determined hypertension. Mean AP and its dynamics during 4 hours post-injection are shown in Table 3. Compound PP-09 (20 mg/kg) had practically no effect on AP and heart rate in normotensive Wistar-Kyoto rats. By contrast, in SHR it induced a 30% decrease in AP 30 min postinjection, and after 3 h AP decreased by 40% in comparison with the initial value, reaching the same AP level as in normotensive rats. A 12-15% decrease in heart rate was statistically insignificant.

From the results obtained it can be concluded that:

- compound PP-09 exhibits high biochemical and physiological activity both *in vitro* and *in vivo*;
- similarly to other "classic" ACE inhibitors [10,13], compound PP-09 effectively reduces AP in SHR and has no effect in normotensive rats;
- compound PP-09 produces a short-term inhibitory effect on tissue ACE (*ex vivo* studies) and prolonged effect on AP in SHR. This indicates a marked difference between biochemical and physiological ACE-dependent mechanisms of AP regulation.

Thus, compound PP-09 is a new antihypertensive preparation (an ACE inhibitor) that requires detailed pharmacological investigations.

The study was supported by the Russian Foundation for Basic Research (grant No. 96-04-48366).

TABLE 3. Dynamics of AP in Normotensive Rats (NTR) and SHR after Injection of Compound PP-09 ($M\pm m$, n=5)

Animals	AP, mm Hg	
	NTR	SHR
Initial	107.8±4.0	166.7±8.8
Before injection	120.4±9.6	165.9±7.2
Time after injection, min:		
30	106.0±1.9	131.6±15.5 (-21.0)
60	106.8±3.7	120.4±12.2* (-27.7)
90	103.3±4.5	116.9±10.1* (-29.9)
120	101.5±6.3	106.5±6.0* (-36.1)
180	104.9±5.2	98.7±6.4* (-40.9)
240	107.9±7.4	105.7±7.4* (-36.5)

Note. Decrease in AP (%) compared with the baseline level is shown in parentheses. *p<0.05 compared with the initial AP.

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