Synthesis and Hemodynamic Effects of a New Endothelin-Converting Enzyme Inhibitor

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A new endothelin-converting enzyme inhibitor PP-35 including N^{α}-benzylsuccinyl group and Leu-Trp-OH dipeptide is synthesized. Similarly to phosphoramidon substance PP-35 abolishes elevation of systemic blood pressure and heart rate decrease in normotensive rats in response to bolus injection of big endothelin-1. Hemodynamic responses to endothelin-1 remain unchanged.

Key Words: *endothelin; endothelin-converting enzyme, inhibitors; hemodynamics*

Endothelins (ET) [16] represent a new class of physiologically active compounds. ET-1 and its analogs ET-2 and ET-3 are potent vasoactive factors.

The contribution of ET to the regulation of endothelial function, vascular tone, and, under pathological conditions, into the development of cardiovascular diseases is the object of much attention [9]. ET are involved into pathogenesis of some forms of essential hypertension, renal ischemia, and subarachnoidal hemorrhage. They play an important role in myocardial infarction, arrhythmias, and cerebrovascular disorders [13].

Biochemical factors involved into ET-1 production in endothelial and secretory cells have been described. Active peptide is formed from its precursor big ET-1 (BET-1) due to the action of ET-converting enzyme (ECE). ECE belongs to membrane-bound proteins that participate in postsecretory processing of peptide hormones and neuropeptides. ECE is similar to neutral endopeptidase 24.11 [15], the prototype of the zinc-dependent metallopeptidase family. Both enzymes are sensitive to phosphoramodon, a classical metalloproteinase inhibitor.

Some ECE inhibitors were synthesized. They are based on known metalloproteinase inhibitors (retro-analog of thiorphan [10]) or contain complexing groups (hydroxamic and phosphomic acid derivatives [4]). Thiol ECE inhibitors not related to phosphoramidon, which inhibited activity of partially purified enzyme, were also synthesized [6]. The synthetic ET-1 analog (D-Val22)ET-1(16-38) inhibits ECE *in vitro* and blocks dopamine release from rat brain *in vivo* [11]. ECE inhibitors were also found among plant or microbial sources [12,14].

We synthesized a new ECE inhibitor that contains the N^{α}-carboxyalkyl complexing group and experimentally studied its modulating influence on the circulatory effects of ET peptides.

MATERIALS AND METHODS

When creating new ECE inhibitor we took into account the structure of an enzyme active site, where terminal amino acid carboxyl group of the inhibitor forms an ionic bond with positively charged recognition site of the enzyme, while other carboxyl group interact with catalytically active zinc ion. Similarly to the angiotensin-converting enzyme inhibitors PP-08 and PP-09 [1,2], the new ECE inhibitor possesses a Zn^{2+}-binding site in the N-terminal region. The C-terminal region is identical to that of phosphoramidon, a standard ECE inhibitor [3] (Fig. 1).

The required substance was synthesized using an original approach of peptide alkylation with N-ben-

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zylimide maleate. Initially, a mixture of diastereomers N-(1-benzyl-3-succinyl)dipeptides heterocyclyc conjugates is formed. These intermediates contain a carboxyalkyl group in an implicit form, since it is included into the imide cycle. It can be hypothesized that this cyclic substance possesses high inhibiting activity due to easy hydrolysis of the imide cycle after binding to the enzyme. The specificity of the ECE-inhibiting effect of PP-35 is determined by the peptide region identical to that of phosphoramidon (-Leu-Trp-OH).

Upon the synthesis of the initial dipeptide, L-tryptophan potassium salt was acylated with N-tret-butoxycarbonyl-(Boc)-L-leucine N-hydroxysuccinimide ester, and protected dipeptide was deblocked with a hydrochloric acid-acetic acid mixture. Alkylation of the dipeptide H-L-Leu-L-Trp-OH in alkaline medium with N-benzylmaleate and subsequent purification yielded the cyclic conjugate PP-35, a mixture of diastereomers in the form of crystal powder. Molecular formula of the compound is $C_{29}H_{33}N_3O_5$. For animal experiments the preparation was dissolved in ethanol and diluted with physiological saline.

Hemodynamic reactions were studied on Wistar rats 1 day after implantation of catheters into the femoral artery for blood pressure (BP) and heart rate (HR) recording and into the jugular vein for infusions. Hemodynamic parameters in alert rats were recorded using a Statham pressure transducer and imputed into a computer with 512 Hz digitalization rate. After stabilization of BP and HR (~40 min), the inhibitor or physiological saline was infused in a bolus followed 3 min later by infusion of BET-1 or ET-1 (Bachem Biochemica). Hemodynamic parameters were recorded for 60 min. The data were processed using nonparametric Mann-Whitney test.

The composition of reaction mixtures and identity of products were controlled by thin-layer chromatography on silica-coated glass plates (Merck) in the following solvent systems: benzene:acetone:acetic acid (100:50:1) and chloroform:methanol:water (85:14:1). The substances were detected with ninhydrin (5% solution in butanol, 110°C) and benzidine reagent after chlorination. Optical rotation was measured on a Perkin-Elmer 241 polarometer in 10-cm-long cuvettes. Protected amino acids were purchased from Fluka and Reanal. Phosphoramidon was from Sigma.

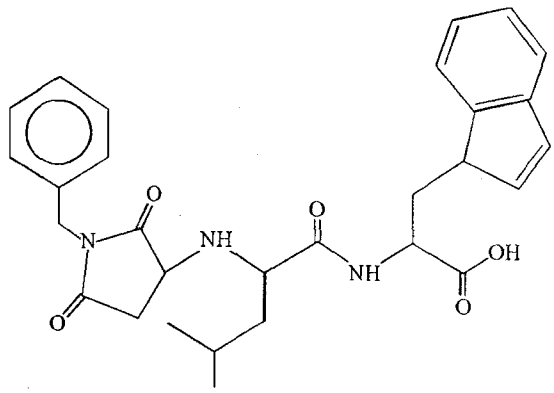

Fig. 1. Structure of PP-35.

RESULTS

Intravenous infusion of ET-1 usually causes BP rise due to its potent vasoconstrictory effect. The ET-1 precursor BET-1 induces a weaker hemodynamic response; the effect is due to ECE-catalyzed cleavage of BET-1 followed by the formation of ET-1. Biological tests with BET against the background of ECE inhibitors allow one to evaluate ECE activity and characteristics of ECE inhibitors [5].

In our experiments bolus infusion of BET-1 to alert rats induced a progressive rise of BP and reduction in HR during a 40-50-min period (Table 1). Neither phosphoramidon (standard preparation), nor PP-35 had any effect on hemodynamic responses to BET-1. Thus, PP-35 abolished the shifts of BP and HR induced by BET-1.

In control experiments the active peptide ET-1 increased BP and decreased HR (Table 2) peaking 2-3 min postinfusion. ET-1 infused against the background of phosphoramidon and PP-35 generated the same response as without inhibitors. Additionally, we studied the effect of the angiotensin-con-

TABLE 1. Effect of ECE Inhibitors on Hemodynamic Effects of BET-1 (5×10^{-8} mol/kg) in Wistar Rats ($M\pm m$, n=6)

Preparations	Initial values		Peak effects	
	BP	HR	BP	HR
BET-1	101.3±9.5	342.4±29.1	110.6±8.8*	316.1±31.7*
Phosphoramidon, 5×10^{-6} mol/kg+BET-1	112.2±7.6	391.2±36.4	114.0±6.8*	377.8±22.0*
PP=35, 5×10^{-6} mol/kg+BET-1	102.6±2.7	331.8±44.3	103.3±3.1*	337.5±29.7

Note. *p<0.05 compared with the initial values. Here and in Table 2: the mean BD value is presented.

TABLE 2. Effect of ET-1 (4×10^{-10} mol/kg) and ECE Inhibitors on BP and HR in Wistar Rats ($M\pm m$, $n=6$)

Preparations	Initial values		Peak effects	
	BP	HR	BP	HR
ET-1	118.7±13.4	384.4±62.7	131.1±12.8*	331.1±38.8*
Phosphoramidon, 5×10^{-6} mol/kg+ET-1	109.2±11.5	357.1±64.4	121.4±9.9*	342.0±71.6
PP-35, 5×10^{-6} mol/kg+ET-1	115.2±11.1	373.5±22.6	127.4±8.8*	304.9±48.8*
PP-09, 5×10^{-6} mol/kg+ET-1	117.2±3.7	311.0±27.7	120.0±3.5	300.1±30.5

Note. *$p<0.01$ compared with the initial values.

verting enzyme inhibitor PP-09, which possesses no ECE-inhibiting properties [3].

Much evidence points to the involvement of angiotensin II into physiological effects of ET peptides. Therefore, angiotensin-converting enzyme inhibitors (enalapril, captopril, and fosinoprol) reduce blood concentration of ET-1 and diminish hypertensive responses to ET-1 in humans [7,8]. PP-09, a new angiotensin-converting enzyme inhibitor, abolished BP rise and HR decrease induced by ET-1 (Table 2).

Our findings suggest that PP-35, a new synthetic N^{α}-carboxyalkyl ECE inhibitor, abolishes hemodynamic responses to ET precursor BET-1 without modifying the effect of active peptide ET-1. The new ECE inhibitor will be tested in other experimental models.

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REFERENCES

1. O. A. Gomazkov, V. F. Pozdnev, E. V. Kalinina, *et al., Byull. Eksp. Biol. Med.*, **122**, No. 9, 279-281 (1996).
2. E. V. Kalinina, V. F. Pozdnev, N. V. Komissarova, and O. A. Gomazkov, *Biokhimiya*, **62**, No. 3, 247-250 (1997).
3. K. Barnes, K. Shimada, M. Takahashi, *et al., J. Cell. Sci.*, **109**, 919-928 (1996).
4. R. Bihovsky, B. Levinson, R. Loewi, *et al., J. Med. Chem.*, **38**, 2119-2129 (1995).
5. R. Corder and J. Vane, *Biochem. Pharmacol.*, **49**, 375-380 (1995).
6. P. Deprez, J. Guillaume, J. Dumas, and J.-P. Vevert, *Bioorg. Med. Chem. Lett.*, **6**, 2317-2322 (1996).
7. P. Dispaquale, L. Valdes, V. Albano, *et al., J. Cardiovasc. Pharmacol.*, **29**, 202-208 (1997).
8. S. Galatiusjensen, H. Wroblewski, C. Emmeluth, *et al., Cardiovasc. Res.*, **32**, 1148-1154 (1996).
9. G. Gray and D. Webb, *Pharmacol. Ther.*, **72**, 109-148 (1996).
10. P. Kukkola, N. Bilci, W. Kozak, *et al., Bioorg. Med. Chem. Lett.*, **6**, 619-624 (1996).
11. A. Morita, M. Nomizu, M. Okitsu, *et al., FEBS Lett.*, **353**, 84-88 (1994).
12. A. Patil, A. Freyer, D. Eggleston, *et al., J. Nat. Prod.*, **60**, 306-308 (1997).
13. R. Rhoten, Y. Comair, D. Shedid, *et al., J. Neurosurg.*, **86**, 101-108 (1997).
14. Y. Tsurumi, H. Uede, K. Hayashi, *et al., J. Antibiot.* (Tokyo), 48, 1066-1075 (1995).
15. A. Turner and L. Murphy, *Biochem. Pharmacol.*, **51**, 91-102 (1996).
16. M. Yangisawa, H. Kurihara, S. Kimura, *et al., Nature*, **332**, 411-415 (1988).