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In vitro Studies on Angiotensin-I Converting Enzyme (ACE I) Inhibitory Activity of Short Synthetic Peptides on Smooth Muscle Preparations (Rat Ileum)

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Introduction

It is well known that in the renin-angiotensin system (RAS) ACE I has a significant role, where it acts as a dipeptidyl-carboxypeptidase and cleave the *C*-terminal dipeptide His[9]-Leu[10] from Angiotensin I (AT I) 1 to produce the vasoconstrictor peptide Angiotensin II (AT II) [1]. In order to avoid this negative effect on the living organisms with high blood pressure, it is necessary to search for new inhibitors of ACE I. To shed light on the effect of chemical structure of different inhibitors it is necessary to investigate ACE I isolated from different sources, as well as purified enzyme and segments of rat ileum.

Nowadays, an increasing number of ACE inhibitory peptides have been isolated and identified from the hydrolysates of various natural sources. Most of them are screened based on their *in vitro* ACE inhibitory activities, however the *in vivo* antihypertensive effects of most of them have not been confirmed.

The purpose of the present study was to investigate *in vitro* effects of low molecular weight synthetic peptides obtained by SPPS as novel inhibitors of Angiotensin-I converting enzyme, for better understanding of the relationship structure/activity and the intermolecular-interaction mechanism.

Results and Discussion

The synthesis of each of the target peptides: H-Val-Ala-Trp-OH, H-Val-Ala-Pro-OH, H-Leu-Ala-Pro-OH, H-Leu-Lys-Pro-OH, H-Ile-Ala-Lys-OH, was realized by Fmoc strategy of SPPS. The TBTU/DIPEA method was used for coupling of each amino acid. For the synthesis of the peptides with free *C*-terminal group 2 chlortrityl chloride resin (ClTR) and Wang resin were used by us successfully. In the Figure 1 is shown the reaction scheme of the synthesis of H-Leu-Ala-Pro-OH.

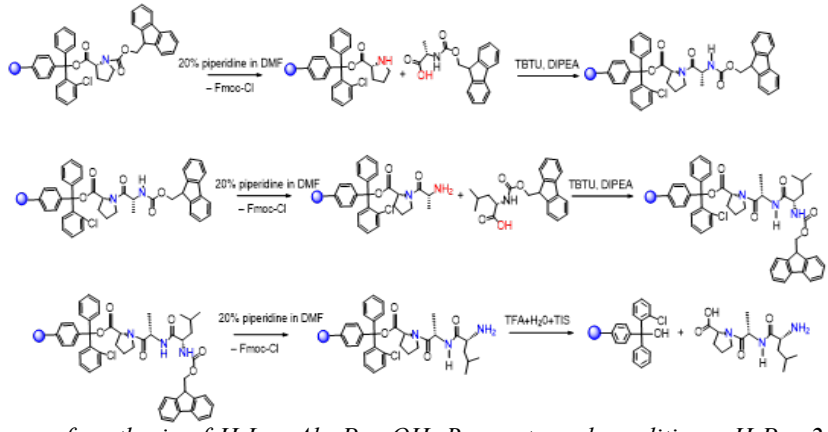

Fig. 1. Scheme of synthesis of H-Leu-Ala-Pro-OH. Reagents and conditions: H-Pro-2-ClTrt resin, Fmoc-Ala, Fmoc-Leu, HOBT, TBTU, DIPEA, TFA / triisopropylsilane / H_2O (95: 2.5: 2.5), room temperature, 1.5 h reaction time.

Table 1. Calculated EC_{50} values for rat ileum contractions after different concentrations of AT-I. EC_{50}ratio is the ratio of EC_{50} in the presence of inhibitor divided by EC_{50} of agonist (AT I) alone.

	Control –AT I EC_{50}	*EC_{50} ratio*			
Concentration		*0.01 μM*	*0.1 μM*	*1 μM*	*10 μM*
LAP	11.0^{-8}		3.562	4.55	
VAP	1.5^{-8}			1.777	5.947
LKP	5.5^{-8}		3.075	3.506	
IAK	3.04^{-8}		2.914	7.573	
VAW	1.98^{-8}		3.494	3.995	
LIS	4.09^{-8}	8.11	18.14		
Average±SD	$6.2^{-8} \pm 4.3$				

Spontaneously hypertensive rats (SHR) are a well- known animal model to study the pathophysiology of hypertension and have been widely used in scientific research [2,3]. The most widely used method described in the literature for determination the inhibitory activity of different compounds on ACE activity in isolated organs is the introduction of two or three different concentrations AT I in the incubation medium in presence or absence of inhibitor. For the purpose of our research, we performed preliminary experiments with rat ileum, replacing the single administration of AT I with cumulative (ATI 10^{-9}M – 10^{-6}M). We have found that this kind of preparation is sufficient sensitive to AT I and can be used for the purpose of our study. The cumulative input allows us to be more detailed monitoring the inhibitory effect of the peptides at all concentrations used AT I.

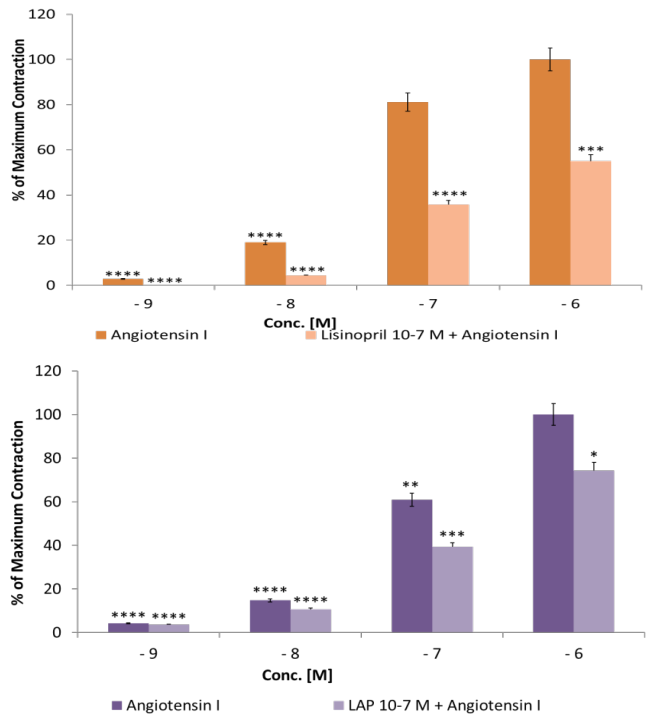

Fig. 2. Effects of Lisinopril and LAP on the angiotensin-induced contractions of rat ileum segments.

The effect of different ACE inhibitors on the contractile response of rat ileum after AT I cumulative application (reported by EC_{50}) is most significantly expressed by the ratio of EC_{50} calculated of the control curve (without inhibitor) and EC_{50} determined of the curve in the present of investigated peptide (so-called EC_{50} ratio) (Table 1).

All investigated tripeptides Val-Ala-Pro (VAP), Val-Ala-Trp (VAW), Leu-Lys-Pro (LKP), Leu-Ala-Pro (LAP), Ile-Ala-Lys (IAK) tested at 2 concentrations decreased dose-dependently AT I-induced contractions of the segments (Figure 2). This suggests that most likely these compounds specifically inhibit ACE activity.

The contractions induced by cumulatively applied AT1 (10^{-9}M – 10^{-6}M) were considered as control values and compared to those obtained after a 2 min incubation of the muscle segments with the peptides tested (10^{-7}M,10^{-6}M). Lisinopril in the same concentrations was also used like a test compound.

Studies have shown that the C-terminal aminoacid of an inhibitory peptide highly influences its binding to ACE I. It is established that potential ACE inhibitors are comprised of hydrophobic (aromatic or branched side chains) amino acid residues at the C-terminus where the utmost preferred aminoacid is proline [4].

Our results confirm that hydrophobic C-terminal proline in position 3 is very important for the inhibitory effect of the new synthetic compounds. Furthermore, the replacement in the second positition of positively charged amino acid lysine (LKP) with hydrophobic alanine significantly increases the inhibitory activity of the new compound (LAP). The combination Ala^2-Pro^3 leads to an enhanced inhibitory effect. The combination Ala^2-Pro^3 enhances the inhibitory effect. Differences between the peptides inhibitory potency were observed when comparing their effect on biological object - smooth muscle preparations (rat ileum) due to AT-I induced contractions. Differences between the peptides inhibitory potency were observed comparing their effects on AT I induced smooth muscle contractions (rat ileum). It is important to note that, in addition to direct inhibition of ACE, other physico-chemical characteristics (e.g., transport through the cell membrane) are responsible for differences in the inhibitory activity of different peptides-the short peptide sequences are easily absorbed into the blood circulation and retain their activity. An understanding of the correlation between the peptide sequence and its bioactivity as an inhibitor is crucial in generating the potentially active peptide ACE inhibitors.

Acknowledgments

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