

In vitro Effects of Bioactive Tripeptides, Ile-Pro-Pro and Val-Pro-Pro on Angiotensin Converting Enzyme (ACE) and Chymase

Lehtinen R¹, Jauhiainen T^{1,2}, Korpela R^{1,2}, and Vapaatalo H^{1*}

¹Institute of Biomedicine, Pharmacology, 00014 University of Helsinki, Finland; ²Valio Ltd, R & D, Helsinki, Finland
E-mail: risto.lehtinen@helsinki.fi

Introduction

Hypertension is a major risk factor for cardiovascular diseases and a worldwide health problem with serious complications, as well as significant cost to the society. Endothelium, the inner, one cell layer of the vascular wall regulates the vascular functions – constriction and relaxation – i.e. stiffness of the arteries. Healthy endothelium is also antithrombotic while atherosclerotic plaques in the vascular wall act as prothrombotics. In addition to medical prevention and treatment of cardiovascular diseases, life-style factors, such as nutrition, physical activity, weight reduction and abstinence from smoking have considerable impact in this respect.

Milk products and dairy proteins have blood pressure (BP) lowering effects [1,2]. Several milk peptides have antihypertensive effects [3] which have been related to their ACE inhibitory activity. Ile-Pro-Pro and Val-Pro-Pro have also beneficial effects on vascular wall in experimental models [4] and clinical interventions [5].

The aim of the present study was to evaluate in more detail the effects of Ile-Pro-Pro and Val-Pro-Pro on pure, commercial angiotensin II forming enzymes ACE type I, chymase and cathepsin G and in biological preparations.

Results and Discussion

ACE type I (Sigma-Aldrich) was inhibited by Ile-Pro-Pro and Val-Pro-Pro at micromolar concentrations (Fig. 1). IC₅₀ for Ile-Pro-Pro was 0.7, 1.9 and 6.2 μM, and for Val-Pro-Pro 1.4, 3.1 and 13 μM depending on the substrate (Hippuryl-His-Leu concentration 1, 3.3. and 10 mM, respectively) indicating a competitive type of inhibition. Pro-Pro showed inhibitory effect first at millimolar concentrations, while Pro was inactive. Captopril was the reference compound. Its IC₅₀ values were 4.4, 6.4 and 45 nmol, respectively.

Chymase and cathepsin G (Biomol International LP) were not inhibited by the tripeptides significantly.

Biological preparations, mesenteric arterial rings from spontaneously hypertensive rats (SHR) and their normotensive controls, Wistar-Kyoto rats (WKY) were used to show the relevance of ACE inhibition in arterial wall (3). The preparations were incubated for 16 h at +4°C with Ile-Pro-Pro (1 mM) whereafter the contractions/relaxations were tested in cuvettes at 37°C. The angiotensin I induced contractions in WKY rats were clearly reduced by Ile-Pro-Pro preincubation while those to angiotensin II remained unaltered. (Fig 2).

When bradykinin was used as vasorelaxant, preincubation with Ile-Pro-Pro (1 mM) improved the relaxation in SHR but not significantly in WKY (Fig 3).

In conclusion, both the enzymological and bioassay findings support the theory that Ile-Pro-Pro/Val-Pro-Pro

act favourably on blood pressure and arterial stiffness [5,6] mainly by competitive inhibition of ACE in the vascular wall.

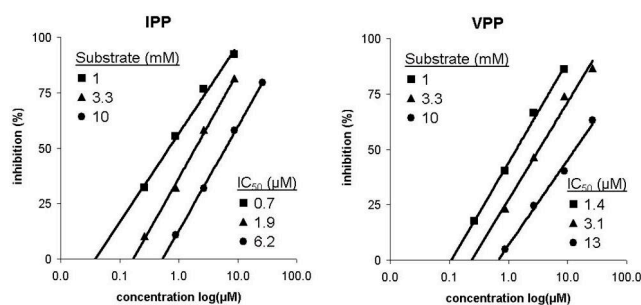


Fig. 1 Competitive inhibition of ACE I by tripeptides Ile-Pro-Pro and Val-Pro-Pro

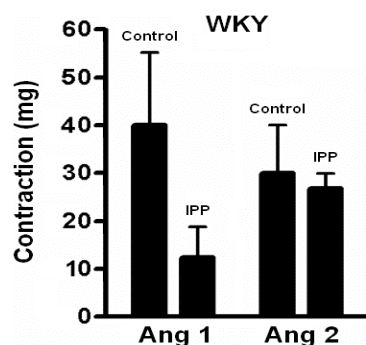


Fig. 2 Preincubation with Ile-Pro-Pro (1 mM) reduces angiotensin I but not angiotensin II contraction of rat mesenteric artery rings

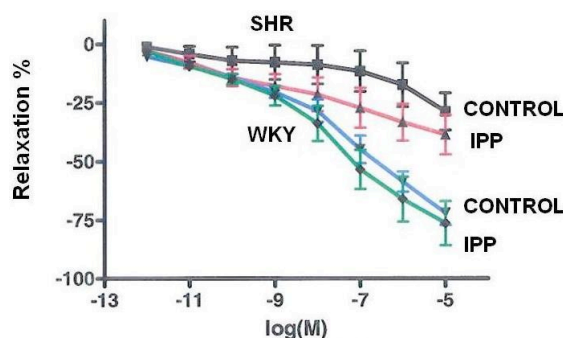


Fig. 3 Preincubation with Ile-Pro-Pro (1 mM) improves bradykinin induced relaxation of hypertensive rat mesenteric artery rings

Acknowledgments

We are grateful to Anneli von Behr for the skillful technical assistance in the biological studies.

References

1. McCarron, D.A., Morris, C.D., Henry, H.J., and Stanton, J.L. (1984) *Science*, **224**, 1392-1398.
2. Burke, V., Hodgson, J.M., Beilin, L.J., Giangiulioi, N., Rogers, P., and Puddey, I.B. (2001) *Hypertension*, **38**, 821-826.
3. FitzGerald, R.J., Murray, B.A., and Walsh, D.J. (2004) *J. Nutr.* **134**, 980S-988S.
4. Sipola, M., Finckenberg, P., Korpela, R., Vapaatalo, H., and Nurminen, M.-L. (2002) *J.Dairy Res.* **69**, 103-111.
5. Jauhiainen, T., Vapaatalo, H., Poussa, T., Kyrönpalo, S., Rasmussen, H., and Korpela, R. (2005) *Am. J. Hypertens.* **18**, 1600-1605.
6. Jauhiainen, T., Rönback, M., Vapaatalo, H., Wuolle, K., Kautiainen, H., and Korpela, R. (2007) *Int. Dairy J.* **17**, 1209-1211.