Although angiotensin I–converting enzyme (ACE) is well into middle age (1) considering the years passed since its discovery, it still provides investigators with novelties. (Of course, the enzyme has not changed in essentials in 600 million years since it arose by gene duplication [2], but its image keeps changing.) The article by Azizi et al. (3) in this issue of *The Journal* adds another facet to ACE research and possibly additional applications of its inhibitors.

Since the discovery of ACE as a factor that converted angiotensin I (Ang I) to II, and as an inactivator of bradykinin by the same peptidyl dipeptidase action (1), it turned out to be a widely distributed enzyme, mostly bound to plasma membrane, which cleaves peptides at protected (substance P) or unprotected (enkephalin) COOH termini and even at NH_2 terminus (luteinizing hormone releasing hormone [LHRH]) (1, 4). The development of ACE inhibitors led to their successful clinical applications (5).

Somatic ACE contains active N- and C-domains (1). The C-domain incorporates a transmembrane anchor peptide, and the domains face the lumen of conduits, whether in blood vessels, intestine, or proximal tubules. The two domains have a high degree of homology at the active centers (2), but differ in some properties. Cl^- activates the N-domain less and kinetics of inhibition of the two active centers are not identical. While both domains cleave bradykinin, Ang I, and substance P, the rate of hydrolysis can be dissimilar with each active site; for example, Ang I is converted about three times faster by the C-domain (6), and at low Cl^- the N-domain cleaves LHRH 12 times faster (4).

Recently, a short active ACE having only an N-domain was recovered from human ileal fluid. It is probably a product of protease(s), but resistant to degradation by the enzyme(s), which cleaves the bridge section connecting the domains. Its activity resembles that of the recombinant ACE with a single, active N-domain (7).

Azizi et al. (3) used captopril, which has a higher affinity for the N-domain active center, in their studies on *N*-acetyl-Ser-Asp-Lys-Pro (Ac-SDKP). The peptide has a protected NH_2 terminus and proline at COOH terminus, thus, it should be resistant to the common, garden variety amino- and carboxy-peptidases. It is released endogenously from the precursor protein thymosin β_4 in bone marrow cells and is a negative regulator of stem and other cells; by blocking G_1-S transition, it blocks DNA synthesis. The peptide is tested clinically to ameliorate the toxicity of some cancer chemotherapeutic agents (3), and, as suggested previously, is cleaved in human plasma by the N-domain of ACE (8). Studies on the kinetics by recombinant mutant ACE showed that Ac-SDKP is hydrolyzed 50 times faster by the N-domain than by the C-domain, with a specificity constant approaching that of Ang I (6). A single oral dose of captopril raised the level of endogenous Ac-SDKP in circulation 5.5-fold in volunteers, from 3 pmol/ml to a peak of 18 (3). The administered captopril also inhibited the slow hydrolysis of Ac-SDKP in aliquots of the subjects' plasma in vitro. The peptide has a 4.5-min half-life in circulation, thus, it is probably released continuously. ACE activity is low in plasma but concentrated on plasma membrane of endothelial, epithelial, and neuroepithelial cells (1). ACE should cleave this substrate primarily on cell membranes, and not in plasma, as shown decades ago by Vane with Ang I (1). The inhibition of ACE in plasma samples can be an indicator of the inhibition obtained in tissues.

Besides the suggested potential use of ACE inhibitors in chemotherapy in combination with Ac-SDKP, the authors speculate further. ACE inhibitors can block vascular tissue proliferation, for example, neointima formation, probably by blocking the release of the mitogenic Ang II and prolonging the half-life of bradykinin, which can be antiproliferative. Ac-SDKP is another substrate for ACE where its inhibitors could affect cellular activity. On the negative side, Ac-SDKP may delay regenerative tissue proliferation or potentially cause anemia (3).

Without employing the too frequently used disclaimer, "Undoubtedly, more experiments have to be done before . . .," which can negate the conclusions of a paper, some questions raised by this initial report should be answered. Is the level of circulating Ac-SDKP high enough to affect stem cells or is it a spillover of a locally acting agent? Where is the peptide metabolized in bone marrow, and where do ACE inhibitors act? Blood cells have low ACE activity. Would ACE inhibitors without the SH group of captopril also raise the endogenous peptide level? Nevertheless, it appears also from this report that the active centers, the twin domains of ACE, located in a single peptide chain, can have separate, important functions, some of them shown here for the N-domain.

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References

1. Skidgel, R.A., and E.G. Erdös. 1993. Biochemistry of angiotensin converting enzyme. *In* The Renin Angiotensin System. J.I.S. Robertson and M.G. Nicholls, editors. Gower Medical Publishing, London. 10.1–10.10.

2. Soubrier, F., C. Hubert, P. Testut, S. Nadaud, F. Alhenc-Gelas, and P. Corvol. 1993. Molecular biology of the angiotensin I converting enzyme. I. Biochemistry and structure of the gene. *J. Hypertens.* 11:471–476.

3. Azizi, M., A. Rousseau, E. Ezan, T.-T. Guyene, S. Michelet, J.-M. Grognet, M. Lenfant, P. Corvol, and J. Ménard. 1996. Acute angiotensin-converting enzyme inhibition increases the plasma level of the natural stem cell regulator *N*-acetyl-seryl-aspartyl-lysyl-proline. *J. Clin. Invest.* 97:839–844.

4. Skidgel, R.A., and E.G. Erdös. 1985. Novel activity of human angiotensin I converting enzyme. Release of the NH_2- and COOH-terminal tripeptides from the luteinizing hormone-releasing hormone. *Proc. Natl. Acad. Sci. USA.* 82:1025–1029.

5. Gavras, H., D.P. Faxon, J. Berkoben, H.R. Brunner, and T.J. Ryan. 1978. Angiotensin converging enzyme inhibition in patients with congestive heart failure. *Circulation.* 58:770–776.

6. Rousseau, A., A. Michaud, M.-T. Chauvet, M. Lenfant, and P. Corvol. 1995. The hemoregulatory peptide *N*-acetyl-Ser-Asp-Lys-Pro is a natural and specific substrate of the N-terminal active sites of human angiotensin-converting enzyme. *J. Biol. Chem.* 270:3656–3661.

7. Deddish, P.A., J. Wang, B. Michel, P.W. Morris, N.O. Davidson, R.A. Skidgel, and E.G. Erdös. 1994. Naturally occurring active N-domain of human angiotensin I converting enzyme. *Proc. Natl. Acad. Sci. USA.* 91:7807–7811.

8. Rieger, K.-J., N. Saez-Servent, M.-P. Papet, J. Wdieczak-Bakala, J.-L. Morgat, J. Thierry, W. Voelter, and M. Lenfant. 1993. Involvement of human plasma angiotensin I-converting enzyme in the degradation of the haemoregulatory peptide *N*-acetyl-seryl-aspartyl-lysyl-proline. *Biochem. J.* 296:373–378.

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