Chymase-Dependent Angiotensin II Formation in Human Vascular Tissue

To the Editor:

The recent article by Takai et al1 suggests that human chymase bound to heparin plays a functional role in angiotensin II formation in the presence of natural protease inhibitors such as α1-antitrypsin (AT). This interpretation is based on one experiment (Figure 4) in which the authors use a heparin column to mimic the physiological situation in which chymase is bound to heparin proteoglycans when secreted from a mast cell during the process of degranulation. However, the experimental setup in Figure 4 raises some doubts about the validity of the obtained result and its in vivo significance.

The authors do not mention whether the heparin column was preincubated with α1-AT or chymostatin; immediate washing with PBS (3 times the column volume) would rapidly and extensively remove the inhibitors from the column. In particular, the large molecular inhibitors, such as α1-AT (Mr=50 000), may be equilibrated slower in the column compared with chymostatin (Mr=604), and the Ki value for α1-AT to chymase is considerably lower than that for chymostatin. Therefore, α1-AT may be easily washed out of the column before interacting with the enzyme under the reported experimental condition. After the washing step, a solution containing the substrate (angiotensin I) but not the inhibitors was applied on the column.

In our opinion, the authors should have performed the test in the presence of inhibitors throughout the experiment. The reaction of chymostatin with chymase results in the irreversible inhibition of chymase through the formation of a stable 1:1 complex, which has no hydrolytic activity. However, according to Schechter et al,2 when the ratios of the natural inhibitors α1-antichymotrypsin and α1-AT to chymase concentration were determined, stoichiometries of 4.5 and 5.0, respectively, were obtained instead of unity. They concluded that both α1-antichymotrypsin and α1-AT are better substrates than chymase inhibitors. Thus, to achieve a full inhibition of chymase, one would have to use an inhibitor concentration >5 times molar excess, which would preferably be present throughout the experiment.

The concentration of α1-AT (2 μmol/L) used by the authors is 50 times lower than that of chymostatin (100 μmol/L). In addition, under normal physiological conditions, the concentration of α1-AT (50 μmol/L)3 is also 25 times higher than the concentration of α1-AT (2 μmol/L) used in this experiment. Taken together, these data seem to indicate that further experimentation is required to unravel the physiological function of heparin-bound chymase in the presence of serpins.

Jorma O. Kokkonen, MD
Wihuri Research Institute
Helsinki, Finland

Denan Jin, MD, PhD
Petri T. Kovanen, MD
Ken A. Lindstedt, PhD
Naotaka Shiotia, MD
Department of Pharmacology
Osaka Medical College
Osaka, Japan

Response

We appreciate and acknowledge what Kovanen et al pointed out about our article. They discuss the difference between the results of our experiment, which hypothesized heparin-bound chymase as the biological state of chymase, and the nature of extracted chymase. We agree with the report by Schechter et al1 that was mentioned in the letter. Certainly, α-antitrypsin is a very powerful chymase inhibitor that has a higher affinity for chymase than chymostatin. However, the inhibitory activity of macromolecular inhibitors, such as secretory leukocyte protease inhibitor, and not that of low molecular weight inhibitors, including chymostatin, was affected by heparin.2 Chymase is bound to heparin in the organism, and the purpose of our article was to study the sensitivity of certain inhibitors on the complex. Consequently, the difference between heparin-bound chymase and extracted chymase is important. Previously, our experiment using both extracts and slices of human vascular tissues demonstrated that the activity of extracted chymase was inhibited by α-antitrypsin, whereas chymase activity in slices was not inhibited by α-antitrypsin.3 We think that α-antitrypsin in the reactive solution had difficulty entering into the tissue slices. Our experiment indicated that α-antitrypsin was unable to work on heparin-bound chymase in the heparin column to inhibit the chymase activity. We think that this phenomenon is what actually occurs in tissues.

The concentration of α-antitrypsin that was used in our experiment was 2 μmol/L. The α-antitrypsin concentration in tissue is lower than that in plasma, and α-antitrypsin at a concentration of 2 μmol/L completely inhibits the activity of extracted chymase. Therefore, we do not think the concentration was too low.

When macromolecular substances are used as the substrate for chymase, the decomposition activity changes extensively depending on the presence or absence of heparin. Consequently, to elucidate the role played by chymase in the organism, it is important to study inhibitors and substrate specificity using heparin-bound chymase.

Shinji Takai, PhD
Denan Jin, MD, PhD
Masato Sakaguchi, PhD
Mizuo Miyazaki, MD, PhD

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Petri T. Kovanen, MD Ken A. Lindstedt, Naotaka Shiota and Jorma O. Kokkonen

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