Authors’ Contributions: All authors confirmed they have contributed to the intellectual content of this paper and have met the following 3 requirements: (a) significant contributions to the conception and design, acquisition of data, or analysis and interpretation of data; (b) drafting or revising the article for intellectual content; and (c) final approval of the published article.

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References

Plasma Renin Activity Enzyme-Kinetic Assay: Protection of Angiotensin I from Bacterial Degradation

To the Editor:

Bystrom et al. (1) recently reported substantial peptidase activity in human plasma that had been incubated at 37 °C to generate angiotensin I (Ang I) during the clinical plasma renin activity (PRA) assay. If this result is correct, it would call into question many of the PRA results previously reported by our laboratory and by Quest Diagnostics, especially those with low PRA (≤0.65 µg·L⁻¹·h⁻¹). We believe, however, this observation is most likely an artifact caused by a failure to follow published protocols (2, 3).

Several features of the Ang I degradation reported by Bystrom et al. (1) indicate the possibility of bacterial contamination: (a) the presence of both carboxypeptidase and aminopeptidase-like activity (their Fig. 4C), (b) a lower rate of Ang I degradation during the shorter Ang I–generation times (their Fig. 2), (c) the independence from the PRA, and (d) the lack of any reported indication of specificity by patient or blood-collection site. Bacterial contamination could occur at many sites: from the patient, at the blood-collection site, during separation of the plasma, and in the laboratory, to name a few.

Given that plasma is an excellent incubation medium for bacteria, we have long considered bacterial contamination a potential source of angiotensin peptide degradation during the Ang I–generation step of the clinical PRA assay (2, 3). For that reason, we routinely add neomycin sulfate to plasma samples before carrying out the Ang I–generation step at 37 °C. Bystrom et al. did not do that. Adding neomycin sulfate is like an insurance policy: Most of the time it is not necessary, but sometimes it is essential. We suggest that Bystrom et al. directly test whether adherence to the classic protocol, including adding neomycin, succeeds in reducing or eliminating the problem of sporadic degradation of Ang I observed in their assay. Until then, it would be prudent to restore adding a bactericidal agent to the Ang I–generation step of their clinical PRA assay or to use another means to protect the generated Ang I, such as anti–Ang I antibodies (4) or heat inactivation of protein-containing assay buffers.

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In Reply

We thank Drs. Sealy and Laragh for their thoughts on the nature of the degradation activity against angiotensin I (Ang I) described in our recent report.

During development of the assay, we identified a number of samples in which the 18-h RIA results generated with the Sealey/Laragh method were below the limit of quantification but the liquid chromatography–tandem mass spectrometry (LC-MS/MS) method produced results in the range of 0.3–0.7 µg·L⁻¹·h⁻¹. In these experiments, neomycin sulfate was used in the RIA but not in the Ang I–generation mixture used for LC-MS/MS. These discordant results led us to explore the nature of the degradation phenomena. Because of the concerns raised, we have repeated our experiments and have again examined the difference in our current method with and without the addition of neomycin. A collection of split patient samples that included both the typical (n = 50) and high-degradation phenotypes (n = 23) were tested in our commercial assay with and without the addition of neomycin. We found no statistically significant difference in the loss of the degradation indicator (P = 0.12, pairwise t-test; Fig. 1). In samples with the high-degradation phenotype, Ang I was below the limit of quantification in all but 2 samples. For typical samples, the correlation of the plasma renin activity results for the samples processed with and without neomycin was excellent (m = 1.004; b = 0.02; r² = 0.954).

Profound degradation activity was observed in some plasma samples, even during very short incubation times (≤15 min), and these observations appear incompatible with bacterial growth during Ang I generation. In the case of our plasma renin activity assay, blood samples are carefully collected into sterile Vacutainers, and the plasma fraction is frozen immediately after separation. Our practice of only thawing immediately before analysis provides little opportunity for bacterial growth before the generation step. Even if bacterial contamination were to occur before generation, the presence of neomycin would serve only to prevent additional bacterial growth; it would not address the strong degradation activity that we monitored with the degradation calibrator.

Although we cannot rule out bacterial contamination before generation, we believe it is highly unlikely, especially considering that month over month, approximately 2% of all plasma renin activity samples exhibit this effect. For a growing number of patients (n > 50) from whom we were able to obtain a second sample, the degradation phenotype was observed in the second sample. In these cases, independent samples were drawn days or weeks apart. In addition, high-degradation samples appear to be randomly distributed, with no dependence on geographic region, clinic, or blood-collection center.

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