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LETTER TO THE EDITOR

Response to Letter to the Editor regarding "A microanalytical capillary electrophoresis mass spectrometry assay for quantifying angiotensin peptides in the brain"

Camille Lombard-Banek[1] · Zhe Yu[2] · Adam P. Swiercz[2] · Paul J. Marvar[2] · Peter Nemes[1]

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This communication is provided in response to the letter entitled *Letter to the Editor regarding "A microanalytical capillary electrophoresis mass spectrometry assay for quantifying angiotensin peptides in the brain"* by A. H. Jan Danser and M. Poglitsch. We appreciate the authors' interest in our work [1], which exemplifies microanalytical capillary electrophoresis high-resolution mass spectrometry (CE-ESI-HRMS) as an attractive technology for trace-sensitive peptidomics. With a natural fit for limited amounts of material, CE-ESI-HRMS emerges as a complementary technology to nano-flow liquid chromatography (LC) in the study of important peptides in the brain and is in higher sensitivity than feasible by conventional LC-ESI-HRMS. In our study, we applied this technology for angiotensin peptide detection in mouse brain nuclei.

To address the questions raised in the *Letter*, we would like to first reiterate the focus of the study. This work was designed to develop and evaluate the suitability of a microanalytical CE-ESI-HRMS instrument and an assay based on parallel reaction monitoring HRMS to identify and *relatively* quantify angiotensin peptides in select brain nuclei. The goal was not to determine the derived cellular source of brain angiotensin peptides (peripheral or central). No comparison was made in our report regarding absolute or relative peptide amounts in the paraventricular nuclei (PVN) and subfornical organ (SFO) in relation to the rest of the brain or potential dilution factors upon tissue averaging; the design of our study had precluded the possibility of such a comparison. Based on a linear correlation between signal abundance and concentration (see Fig. 3), CE-ESI-HRMS was found to enable absolute quantification (peptide levels per gram) using external calibration or internal standards. The assessment of sensitivity by CE-ESI-HRMS was also not performed in tissues: 5 amol to 300 zmol lower limits of detection were determined using chemical standards (see Results and Methods sections). Taking in account these factors, it unfortunately makes direct comparisons from our results to values reported in literature using classical methods difficult. This further brings into question the calculations presented in the *Letter*. For example, Fig. 5 reveals less than ~3-fold variance in the total signal abundance for the angiotensin peptides. Considering a broad-range log-log concentration-signal abundance relationship experimentally established in Fig. 3 or by reasonably assuming a linear relationship within a narrow dynamic concentration range, the observed 3-fold change in peptide signal intensity projects to an ~2–3-fold concentration change in peptide levels. This contrasts to the 10-fold changes concluded in the *Letter*.

The *Letter*'s suggestion that background noise potentially confounded the results is in contrast to the fundamentals and established performance metrics of CE-ESI-HRMS. With theoretical plates on the order of hundreds of thousands to a million, CE is known for an exquisite power to separate molecules. Parallel reaction monitoring employs transitions designed to be specific to each of the angiotensin peptides during tandem mass spectrometry, well-known to provide high molecular specificity for detection [2]. Detection leverages an ultra-high resolution orbitrap mass analyzer capable of measuring peptide masses (m/z values) to millidalton–submillidalton level, delivering unparalleled accuracy. Therefore, this bioanalytical technology combines leading specificity and selectivity in detection and identification, efficiently distinguishing signals from noise. We believe that such studies will be paramount to contributing to the advancement of the brain angiotensin peptide research field and broader field of neuroscience.

This article is a response to the 'Letter to the Editor' to be found at https://doi.org/10.1007/s00216-019-02162-w

✉ Peter Nemes
nemes@umd.edu

[1] Department of Chemistry & Biochemistry, University of Maryland, 8051 Regents Drive, College Park, MD 20742, USA

[2] Department of Pharmacology & Physiology, The George Washington University, 2300 Eye Street NW, Washington, DC 20037, USA

Addressing biologically motivated questions, such as the investigation of brain angiotensin peptide dynamics, calls for specialized experimental strategies, study designs, further validation, and replication of experimental results across laboratories. As the authors are well aware, there are several lines of evidence supporting local production of angiotensin in the brain [3, 4], and we agree that continued study is warranted. As also raised in the *Letter*, are peptides of the renin-angiotensin system intrinsically produced by specific brain nuclei, are they receptor-bound from the blood, or maybe a combination of both? Without buffer perfusion, our original study design did not allow for the differentiation of these scenarios, and correspondingly, we were unable to conclude the origin of the peptides, contrasting the assertions made in the *Letter*. Additional experiments with brain perfusion, an angiotensin receptor antagonist, or isolation of the brain cells (i.e., neurons, astrocytes) and microanalysis of angiotensin-derived peptides may provide the necessary insights. Danser and Poglitsch duly suggest the use of liver-targeted angiotensinogen siRNA to suppress angiotensin generation in blood and/or the use of angiotensinogen knockout mice as alternative approaches toward resolving this question.

In conclusion, we thank again A. H. Jan Danser and M. Poglitsch for recognizing the importance of this study to neuroscience. Ultrasensitive microanalysis by CE-ESI-HRMS affords exciting new measurement capabilities to study important peptides (as well as proteins [5] and metabolites [6]) in limited amounts of tissues and biopsies. The recent commercialization of high-sensitivity CE-ESI instruments (e.g., CESI by AB Sciex) makes the technology available to broad user bases. Recent and continuous innovations in technology, like the one presented in this study, can help obtain answers to existing questions and to also foster new types of queries in neuroscience. The interdisciplinary combination of bioanalytical chemistry and neuroscience is critical to advancing our understanding of the brain during states of health and disease and the development of next-generation diagnostics and therapeutics.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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