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LETTER TO THE EDITOR

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

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Lombard-Banek et al. describe a novel microanalytical capillary electrophoresis-coupled mass spectrometry (MS) approach for angiotensin detection in brain nuclei [1]. Previous studies, also applying LC-MS/MS-based methods, reported low (2–15 fmol/g) or even undetectable angiotensin levels in brain samples. Lombard-Banek now suggests that angiotensin is restricted to very small brain nuclei like the paraventricular nucleus (PVN), which when collected together with surrounding brain areas would become too "diluted" to allow detection. A few caveats remain.

First, the authors do not report levels per gram, making it impossible to compare their data with earlier observations. They have dissolved an extract of 200 nL (0.2 mg) tissue in 2 μL injection buffer, and report a lower-limit-of-quantification of 13 amol/20 nL extract or 6500 fmol/g tissue. The quantifiable PVN signals range at least 10-fold, implicating that PVN angiotensin levels reach up to 65,000 fmol/g. This is > 100-fold above the levels in adrenal and kidney [2]. In fact, had such levels been present in 0.2 mg surrounded by 99.8 mg of angiotensin-free tissue, the "dilution" would have resulted in levels of $0.2/100 \times 65{,}000 = 130$ fmol/g, which the classical method (investigating ≈ 100 mg) should have detected easily. In reality, the PVN weight is > 0.2 mg, and thus it is reasonable to assume that the "diluted" tissue levels per gram would have been far above 130 fmol/g. Yet, the classical approach reported levels that were > 2 orders of magnitude lower [2, 3]. Thus, we need to understand why this novel method detects such high levels. Here, employing internal standards and performing an instrument calibration to report actual tissue levels would help.

Second, brain tissue contains blood or blood-derived angiotensin, possibly bound to receptors. Thus, before concluding that there is "local" angiotensin production, the authors should either remove blood, or, preferably (since blood removal will not take away receptor-bound blood-derived angiotensin), treat the mice with liver-targeted angiotensinogen siRNA, thus suppressing angiotensin generation in blood only [4]. Finally, they might consider applying their method to brain samples from angiotensinogen knockout mice to firmly establish that their measurements are not reflecting background noise.

Compliance with ethical standards

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

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A response to this "Letter to the Editor" can be found at https://doi.org/10.1007/s00216-019-02164-8 .

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