Reply to “Letter to the editor: Angiotensin quantification by mass spectrometry”

Mark C. Chappell
Hypertension and Vascular Research Center, Wake Forest University School of Medicine, Winston-Salem, North Carolina

REPLY: Although I appreciate the comments from Poglitsch and colleagues in their letter (5), their assertion that my review concluded that liquid chromatography-mass spectrometry (LC-MS) lacks the sensitivity to quantify endogenous angiotensins and requires exogenous renin for peptide detection is surprising and quite incorrect. I discussed multiple LC-MS studies that quantified angiotensins in plasma and tissues in the sensitivity range of immunoassays (RIA, ELISA) without the addition of renin that included their own LC-MS analysis of mouse plasma and kidney (1, 2, 6). In fact, their ANG II and ANG III values for mouse kidney were included in Table 1 of the review article that describes a validated range of values for angiotensins in plasma, tissues, urine, and cells (1, 2). Perhaps their misunderstanding arose from my comment on Dr. Poglitsch’s quantitation of plasma ANG II by LC-MS in which they added exogenous renin to human plasma (3, 4). I noted that these data were not comparable with those of other MS or RIA studies and that because of the renin addition, the peptide values do not reflect endogenous angiotensins in human plasma (2). Thus it is also surprising that their letter fails to take this opportunity to explain the rationale for addition of exogenous renin to human plasma samples. The other issues presented in the letter concerning assay sensitivity, sample handling, assessment of recovery, and extraction methods were extensively discussed in my review and cite the same set of references included in the review.

I also stated that Dr. Poglitsch provides a commercial LC-MS service (MS-fingerprint, Attoquant) with the capability to quantify 10 angiotensins including ANG I, ANG II, and ANG-(1-7) in a single sample at a sensitivity or detection limit of RIA (2). An important aspect of this service is the potential for a reference laboratory to accurately quantify angiotensins among various tissues and across multiple species, as well as to expand the detection of peptide isoforms beyond the antibody-based RIA or ELISA methods. However, I would urge that the methods used in this particular approach be more transparent (i.e., identity and concentration of the inhibitors used in sample collection, sequence of standards added, sampling handling without addition of renin as standard procedure, extraction techniques) such that a more thorough assessment of the LC-MS method and the resultant data can occur. As a case in point, LC-MS by Dr. Poglitsch revealed a very high plasma ratio of ANG III to ANG II that may manifest from incomplete inhibition of ex vivo sample processing rather than a valid profile of these peptides in the circulation of both female and male mice (6).

My review concluded, in part, that current LC-MS methods that quantify angiotensins and the peptide values reported in the literature are not consistent at this time. This reflects that fact that several published LC-MS studies report plasma or tissue peptide values that are 100- to 1,000-fold higher and often with a higher ratio of ANG II metabolites to the ANG II parent peptide than LC-RIA or other LC-MS studies where the sample collection and extraction techniques are well documented and appropriate (2). Lastly, recognition of accepted values of angiotensin peptides in various biological compartments facilitates our ability to judge optimal methods of quantitation, as well as assess valid alterations in the peptide components of the renin-angiotensin system (2).

DISCLOSURES
No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS
M.C.C. drafted, edited, revised, and approved final version of manuscript.

REFERENCES