Mass spectrometry for the molecular imaging of angiotensin metabolism in kidney

Nadja Grobe, Khalid M. Elased, David R. Cool, and Mariana Morris

Department of Pharmacology and Toxicology, Wright State University Boonshoft School of Medicine, Dayton, Ohio

Submitted 6 October 2011; accepted in final form 2 February 2012

Grobe N, Elased KM, Cool DR, Morris M. Mass spectrometry for the molecular imaging of angiotensin metabolism in kidney. Am J Physiol Endocrinol Metab 302: E1016–E1024, 2012.-To better understand the tissue distribution and activity of enzymes involved in angiotensin II (Ang II) processing, we developed a novel molecular imaging method using matrix-assisted laser desorption ionization-time-of-flight (MALDI-TOF) mass spectrometry. Mouse kidney sections (12 μm) were incubated with 10–1,000 μmol/l Ang II for 5–15 min at 37°C. The formed peptides Ang III and Ang-(1–7) were identified by MALDI-TOF/TOF. A third metabolite, Ang-(1–4), was generated from further degradation of Ang-(1–7). Enzymatic processing of Ang II was dose and time dependent and absent in heat-treated kidney sections. Distinct spatial distribution patterns (pseudocolor images) were observed for the peptides. Ang III was localized in renal medulla, whereas Ang-(1–7)/Ang-(1–4) was present in cortex. Regional specific peptide formation was confirmed using microdissected cortical and medullary biopsies. In vitro studies with recombimant enzymes confirmed activity of peptidases known to generate Ang III or Ang-(1–7) from Ang II: aminopeptidase A (APA), Ang-converting enzyme 2 (ACE2), prolyl carboxypeptidase (PCP), and prolyl endopeptidase (PEP). Renal medullary Ang III generation was blocked by APA inhibitor glutamate phosphonate. The ACE2 inhibitor MLN-4760 and PCP/PEP inhibitor Z-pro-prolinal reduced cortical Ang-(1–7) formation. Our results establish the power of MALDI imaging as a highly specific and information-rich analytical technique that will further aid our understanding of the role and site of Ang II processing in cardiovascular and renal pathologies.

The next step was the development of an in situ tissue imaging approach for the RAS using matrix-assisted laser desorption ionization (MALDI)-MS coupled with tissue incubation with natural peptide precursors. The method combines the advantages of MS detection with anatomic specificity for measurement of enzyme activities. MALDI imaging was established initially for the visualization and in situ identification of molecules present on or near the tissue surface (8, 10, 48, 49). To date, MALDI imaging and profiling of peptides and proteins have been applied to a wide variety of tissues (37). We explored this novel imaging method with the goal of characterizing and localizing RAS enzyme activities involved in renal Ang II processing. Described herein is the power of a new, dynamic MS technique as a highly specific and information-rich analytical approach that is not restricted to the renal RAS but can be used to address a variety of questions related to enzymatic function in biological tissues.

METHODS

Animals and tissue samples. Male C57BL/6 mice were purchased from a commercial source (Harlan, Indianapolis, IN). Animals were housed at 22°C under a 12:12-h light-dark cycle with ad libitum access to water and standard mouse chow (3.0 kcal/g, 40.6% carbohydrate, 5.5% fat, and 22% protein; Harlan-Teklad, Madison, WI). Mice (12–16 wk old) were decapitated, and kidneys were quickly removed and frozen in liquid nitrogen. This procedure from decapitation to freezing of kidneys was accomplished in <30 s. Kidneys were stored at −80°C until they were used for testing. All experimental protocols were approved by the Wright State University Animal Care and Use Committee.

Tissue sectioning. Kidney halves (sagittal) were placed on the cryostat chuck and embedded with a small amount of freezing medium (Triangle Biomedical Sciences, Durham, NC). Sections (12 μm) were cut on a cryostat at −20°C. Kidney sections were thaw-mounted onto precoated indium-tin-oxide coated glass slides and then desiccated at room temperature for 15–20 min followed by incubation with Ang II.

In situ enzyme activity. Kidney sections were incubated with 10–1,000 μmol/l Ang II (dissolved in water) at 37°C for 5–15 min. In control experiments, sections were autoclaved for 15 min at 121°C to
Ang II peptide processing in renal medulla and cortex punch biopsies. Frozen kidneys were sliced coronally (1-mm sections) using an acrylic slicer matrix. Biopsies from medulla and cortex were collected using a prechilled 1.5-mm stainless-steel punch (Integra Miltex, York, PA). Three medulla and three cortex tissue pieces from one kidney were combined and homogenized as 10% suspensions in Lysis-M EDTA-Free buffer (Roche Applied Science) using a precells 24-tissue homogenizer (Bertin Technologies, Montigny-le-Bretonneux, France). The homogenate was centrifuged at 10,000 g for 5 min to remove cellular debris. Total protein content was determined in the supernatant using the Bradford protein assay with BSA as a standard (Bio-Rad Protein Assay Reagent). Protein samples (100 µg) were incubated with 1 mmol/l Ang II for 15 min at 37°C. In control assays, 1 mmol/l Ang II was incubated without protein in Lysis-M EDTA-Free buffer for 15 min at 37°C. The reaction was acified by adding trifluoroacetic acid (TFA; final concentration 1%) and dried for 4 h using a vacuum concentrator. The extracts were reconstituted in 0.1% TFA and loaded onto a SepPak C18 cartridge (Waters) that had been preconditioned with two 1-ml portions of 80% methanol containing 0.1% TFA and two 1-ml portions of 0.1% TFA. The cartridge was washed twice with 1 ml of 0.1% TFA and 1 ml of water. Peptides were eluted with 80% methanol containing 0.1% TFA, dried with a vacuum concentrator, and resuspended in 90% acetonitrile containing 0.3% TFA. Samples were directly injected (flow rate of 200 µl/h) into an HCT Ultra ion trap with an electrospray ion source (Bruker Daltonics). For detection of Ang peptides, the ion trap was operated with positive polarity in standard enhanced scan mode from m/z 50 to 3,000. The ion source parameters were set as follows: nebulizer pressure, 10 psi; dry temperature, 300°C; and dry gas flow, 5 l/min.

\[A\] quick removal and rapid freezing of organs after collection, 2) avoidance of localized tissue warming and contamination of tissue-cutting surface with freezing medium, 3) care in applying the neptide solution such that the tissue is not damaged, 4) minimal movement of slides during incubation and transfer to desiccator, and 5) uniform matrix coating.

**MALDI IMAGING OF RENAL ANGIOTENSIN METABOLISM**

**Fig. 1.** Optimization of matrix-assisted laser desorption ionization (MALDI) imaging for angiotensin (Ang) III and Ang-(1→7) in kidney sections. Sections (12 µm) were obtained from fresh frozen mouse kidneys. Peptide standards were spotted onto kidney sections. A: dependency of Ang III signal intensities on matrix coating. B: dependency of Ang-(1→7) signal intensities on matrix coating. C: light microscopic image of matrix coating. D: MALDI dose-response curve for Ang III. E: MALDI dose-response curve for Ang-(1→7).
RESULTS

Method optimization of MALDI imaging for Ang peptides in kidney. MALDI has been widely used for the detection of peptides and proteins in tissues and biological fluids (3, 8, 28, 34). In our studies, the MALDI laser is set to automatically screen tissue sections, producing an ordered array of mass spectra with information on nominal m/z values (8). Pseudocolor images are then created by integrating the m/z peak areas and plotting the relative values using a color scale (48). Our goal was to use MALDI imaging for characterization and localization of Ang II processing in kidney sections. The first step was to establish and validate the method for matrix application and to determine the peptide dose response in tissue sections. To evaluate the dependency of the MS signal intensity on matrix coating, three mouse kidney sections were spotted randomly with 500 ng of Ang III or Ang-(1–7), the two main Ang II metabolites, and spray-coated with increasing amounts of MALDI matrix. Peptide intensity was calculated for each peptide spot and normalized to the entire area of the kidney section. Peptide signals were optimal with 12 or more spraying cycles (Fig. 1, C). To determine the linearity of MS signal intensities, 25–250 ng of Ang III and Ang-(1–7) were spotted randomly onto kidney sections and analyzed by MALDI imaging. Quantitation of signal intensities revealed that MS signals for the peptide standards Ang III and Ang-(1–7) were reproducible and linearly dependent on peptide concentration in a directly proportional manner (Fig. 1, D and E). The assay limit of detection was ~5 ng for all Ang peptides. This is similar to the approach of Nilsson et al. (39) for the drug standard tiotropium.

In situ MALDI imaging of renal Ang II metabolism. Ang II metabolism in kidney sections was documented by measurement of peptide formation, amount, and tissue localization. Mouse kidney sections were incubated with 10 μl of 1,000 μmol/l Ang II for 15 min, followed by analysis using MALDI imaging. The resulting array of mass spectra was screened for newly formed peptides generated on the kidney section from Ang II. Two new peaks with m/z 931 and m/z 899 were detected, consistent with the molecular masses of Ang III and Ang-(1–7), respectively (Fig. 2). These peptides were absent in kidney sections not incubated with Ang II. In addition, the reaction was destroyed by heat treatment, confirming that the processing was of enzymatic origin. The product m/z 931 was localized predominantly in the renal medulla, whereas the second peptide with m/z 899 was detected mainly in renal cortex. An overlay of the MS signals obtained for the two peptide products illustrates their distinct spatial distribution patterns (Fig. 2E). This clear differentiation of Ang III and Ang-(1–7) formation was also detected in 15-min incubations with Ang II peptide spots (1,000 μmol/l) placed randomly across the kidney section (Fig. 2F). A search for other Ang peptide species (Table 1), as expected from further degradation, revealed detection of m/z 552 in renal cortex. This peptide was identified as Ang-(1–4), a biologically inactive tetrapeptide generated from Ang-(1–7) by neprilysin (2).

Identification of metabolites m/z 931, m/z 899, and m/z 552. Figure 2 shows the localization pattern for peptides generated from Ang II but not their precise structure. The next important step was identification and quantification of the Ang II metab-

![Fig. 2. MALDI imaging of Ang II and metabolites generated on kidney sections. A: mass spectrometric (MS) signal for Ang II [mass-to-charge (m/z) 1,046] before incubation. B: MS signal for m/z 931 detected after 15 min of incubation with 1 μmol/l Ang II. C: MS signal for m/z 899 detected after 15 min of incubation with 1 μmol/l Ang II. D: photographic image of a kidney section. E: overlay of both MS signals m/z 931 and m/z 899. F: kidney section spotted 6 times with 1 μmol/l Ang II and incubated for 15 min. Overlay of both MS signals m/z 931 and m/z 899.](image-url)
olites formed in mouse kidney. The chemical nature of the generated peptides was verified by MS/MS, in which the precursor ion of interest is selected and fragmented into ions that are subsequently analyzed. Using the MALDI LIFT-TOF/TOF function of the Bruker Autoflex III MS instrument (50), we first obtained MS/MS spectra for synthetic peptides Ang III, Ang-(1–7), and Ang-(1–4) spotted onto kidney sections (Fig. 3). These MS/MS spectra were identical to the MS/MS spectra of m/z 931, m/z 899, and m/z 552 generated enzymatically in mouse renal medulla and cortex (Fig. 3).

Reproducibility of renal Ang II metabolic assays. To apply the metabolic assays to pathological testing, it was important to quantify peptide levels and establish reproducibility. Peptide formation on consecutive kidney sections obtained from a single animal (intra-animal variation) and kidney sections obtained from different animals (interanimal variation) was evaluated for reproducibility of the newly established MALDI imaging technique. There was little variation for Ang III and Ang-(1–7) forming enzymatic activities within (19.4 ± 1.9 and 13.1 ± 2.0 intensity/area, respectively) or between animals (20.9 ± 1.6 and 12.7 ± 2.0 intensity/area, respectively).

Effect of time and substrate concentration on peptide formation. Distinct Ang III and Ang-(1–7) product profiles were observed in kidney sections incubated with various Ang II concentrations, 10–1,000 μmol/l, for 5 and 15 min (Fig. 4).

At low Ang II concentrations, formation of Ang III increased with incubation time, whereas Ang-(1–7) decreased likely because of a high efficiency of enzymes degrading the formed peptide. At high Ang II concentrations, both Ang III and Ang-(1–7) formation increased with incubation time. In this set of experiments, there was again consistency in the localization pattern: Ang III in the medulla and Ang-(1–7) in the cortex.

Ang III and Ang-(1–7) degradation in kidney. These distinct spatial distribution patterns were further analyzed by a study of peptide degradation. Kidney sections were incubated with 100 μmol/l Ang III or 100 μmol/l Ang-(1–7) for 1–5 min. Ang III was rapidly degraded in renal cortex but was still detectable in medulla after 5 min. Ang-(1–7) was degraded much more slowly, and there was no difference between cortex and medulla (Fig. 5).

Complementary studies of Ang II peptide processing. The quality of MALDI-MS signals depends highly on matrix composition and sample preparation (11). MALDI imaging carries the potential risk of ionization biases within a heterogenous tissue (31). To further validate the MALDI imaging results, we examined Ang II processing in renal cortex and medulla, using punch biopsies taken from kidney sections coupled with peptide purification and a complementary mass spectrometric approach. In 100-μl test assays, renal medulla and cortex biopsy homogenates (100 μg) were incubated with 1 mmol/l

Fig. 4. Dependency of Ang III and Ang-(1–7) formation on incubation time and Ang II substrate concentrations. Kidney sections were incubated with varying concentrations of Ang II (10–1,000 μmol/l) for 5 and 15 min. Metabolism of Ang II and formation of Ang III/Ang-(1–7) peptides were analyzed by MALDI imaging. A: presence of Ang II after incubation of kidney sections with 10–1,000 μmol/l Ang II for 5 and 15 min. B: formation of Ang III after 5 and 15 min of incubation with 10–1,000 μmol/l Ang II. C: formation of Ang-(1–7) after 5 and 15 min of incubation with 10–1,000 μmol/l Ang II. Note that bars are absent for 10, 50, and 100 μmol/l in A and C.
Ang II for 15 min. The incubation mixtures were purified by C18 solid-phase extraction and injected directly into an HCT Ultra ion trap MS. A signal for m/z 931 but not m/z 899 was detected in the medulla enzyme assays, whereas cortex enzyme assays showed a peak only with m/z 899, confirming the MALDI imaging results (Fig. 6).

Identification of Ang III and Ang-(1–7) forming enzymes. APA (EC 3.4.11.7) has been shown to form Ang III through hydrolysis of the Asp1-Arg2 peptide bond of Ang II (33). Three different enzymes, ACE2 (EC 3.4.17.23), PEP (EC 3.4.21.26), and PCP (EC 3.4.16.2), are known to generate Ang-(1–7) from Ang II through COOH-terminal cleavage of the Pro7-Phe8 bond of Ang II (30, 51, 53, 55, 57). We first confirmed activity of recombinant APA, ACE2, PCP, and PEP with Ang II as substrate (Fig. 7) and further characterized Ang II processing in kidney using peptidase inhibitors. Amastatin, a general inhibitor of APA (52), did not prevent Ang III formation under any conditions. Therefore, kidney sections were incubated with 100 or 1,000 μmol/l Ang II for 5 or 15 min, respectively, in the presence of the specific APA inhibitor glutamate phosphonate (GluP) (35). Analysis by MALDI imaging revealed that GluP (3 μmol/l) effectively inhibited Ang III formation ≤60% (Fig. 8A).

Next, two inhibitors were chosen to test for their effects on renal Ang-(1–7) formation: MLN-4760 (MLN), a specific ACE2 inhibitor (14), and Z-polyl-prolnal (ZPP), a PCP and PEP inhibitor (51, 58). At 100 μmol/l Ang II and 5 min of incubation time, Ang-(1–7) formation was inhibited by MLN (1 μmol/l) to 38 ± 1% but not by ZPP (10 μmol/l) (Fig. 8B). A minimal effect on enzyme activity by the PCP/PEP inhibitor suggests ACE2-independent Ang-(1–7) formation at low Ang II concentrations. However, PCP/PEP involvement in Ang-(1–7) formation was more evident with higher Ang II levels (1,000 μmol/l) and at 15 min of incubation time. This is documented in Fig. 8B, which shows that the response to the specific inhibitors MLN and ZPP was switched in the face of higher Ang II levels (1,000 μmol/l). MLN (1 μmol/l) had no effect on Ang-(1–7) formation whereas ZPP (10 μmol/l) produced an inhibition of 80 ± 12% (Fig. 8B). In conclusion, assays that used <100 μmol/l Ang II assessed ACE2 activity, whereas assays that used 1,000 μmol/l Ang II detected PCP/PEP activity.

**DISCUSSION**

This study describes the development of a reliable and highly specific method that uses MALDI imaging for the determination of Ang II metabolites and Ang II processing activity in regions of mouse kidney. MALDI imaging of kidney sections incubated with Ang II detected three peptides with m/z 931, m/z 899, and m/z 552. The two main Ang II products were identified as Ang III and Ang-(1–7) using MALDI-TOF/TOF. The peptide with m/z 552 was assigned to Ang-(1–4), a biologically inactive degradation product of Ang-(1–7). Ang III was localized predominantly in renal medulla, whereas Ang-(1–7) and Ang-(1–4) were detected mainly in cortex. This clear differentiation of Ang II peptide processing was confirmed in incubations with Ang II spotted across the kidney section as well as in a complementary MS approach. Validation of the MALDI imaging technique showed a linear signal dose response for Ang peptides, and only minimal

---

**Fig. 5.** Degradation of Ang III and Ang-(1–7) in mouse kidney sections.

**Fig. 6.** Complementary studies of Ang II peptide processing. A: MS signal for 1,000 μmol/l Ang II incubated for 15 min at 37°C in lysis buffer without kidney protein. B: MS of 1,000 μmol/l Ang II incubated for 15 min at 37°C with medulla protein (100 μg) obtained from kidney punch biopsies. C: MS of 1,000 μmol/l Ang II incubated for 15 min at 37°C with cortex protein (100 μg) obtained from kidney punch biopsies.
variations were observed within and between animals, indicating good reproducibility. Formation of Ang III and Ang-(1–7) was absent in heat-treated kidney sections and dependent on incubation time and Ang II concentrations. Under conditions of high substrate concentration and at longer incubation times, elevated amounts of Ang II metabolites were detected. At short time periods and high Ang II concentrations, lower Ang-(1–7) and Ang III levels were found, which was likely due to a high efficiency of enzymes degrading the formed peptides. Studies of peptide degradation showed that Ang III was more rapidly degraded in renal cortex compared with medulla, whereas Ang-(1–7) was degraded at similar rates in cortex and medulla. The enzyme reactions leading to Ang III and Ang-(1–7) formation were further characterized. We first confirmed activity of recombinant APA, ACE2, PCP, and PEP with Ang II. Use of specific inhibitors GluP (APA inhibitor), MLN (ACE2 inhibitor), and ZPP (PCP/PEP inhibitor) indicated that APA is responsible for medullary Ang III formation, whereas ACE2 and PCP/PEP generate cortical Ang-(1–7).

Ang III and Ang-(1–7) are involved in the regulation of blood pressure and renal function (20). The observed spatial distribution patterns of the Ang II metabolites suggest regionally localized biological actions. Ang III possesses effects and receptor-binding affinities similar to those observed for Ang II, suggesting that this heptapeptide may be equally or even more important than Ang II in some actions, e.g., aldosterone or vasopressin release (60, 63). Indeed, there is a drug development that targets Ang III formation with the idea that it might be a treatment strategy for certain forms of hypertension (5, 6). Our results document an abundance of Ang III formation in the medulla, providing support for a pathway by which Ang III might interact to affect aldosterone and vasopressin.

Ang-(1–7) is thought to oppose the deleterious actions of Ang II, causing vasodilation and antiproliferation and perhaps mediating other beneficial cardiovascular and renal actions (20). Indeed, it has been postulated that ACE2 is renoprotective due to its highly specific action to generate Ang-(1–7) from Ang II (21, 38, 59, 61, 62). In the kidney, the highest levels of ACE2 and the proposed Ang-(1–7) binding site are found in cortical proximal tubules (13, 27, 40, 62). This is consistent with our results suggesting that ACE2-dependent Ang-(1–7) formation occurs mainly in renal cortex.

Using MALDI imaging, there was no evidence for endogenous Ang peptides in kidney. This is likely due to a low abundance along with the effects of fast-acting degradative enzymes. Endogenous Ang levels reported in literature are <500 fmol/g kidney tissue (9, 22), which is below our MALDI-based limit of detection. In addition, matrix and biological background interferences are known to impair ionization of low-abundance molecules. Mass spectrometry coupled with electrospray ionization and liquid chromatography was the only method to achieve sensitivities needed to detect minute amounts of endogenous Ang peptides in biological tissue (12, 36). The benefit of the MALDI imaging approach is that it does not require pooling of samples and still allows for the localization of enzyme activity within biological specimens. The advantage of the activity method is that a small concentration of active enzyme (ng amounts) is sufficient to convert a substrate into product at levels detectable by MALDI imaging.

Besides limitations in detection sensitivity and sampling efficiency, the imaging approach carries other potential risks related to sample preparation, postmortem degradation of peptides and proteins, ionization bias, and lateral diffusion of...
analytes during incubation (19, 24, 31, 45, 54). Using multiple conditions and treatment, these issues have been addressed and managed in our study. For matrix application, we developed a simple yet reliable method as described in METHODS. An automated system is not an absolute requirement. Moreover, compliance to a standardized sample workup protocol and strict observation of postmortem processing times minimized differences and irregularities of MS signals. Although we cannot exclude some postmortem degradation that could change endogenous active enzyme forms, this does not appear to be an important issue given the reproducibility of the results. The problem of ionization biases within heterogenous tissue such as the kidney can be excluded in our study since signal intensities for all Ang peptides were equal over the entire kidney section (Figs. 2A and 5). Scale bars in these figures are set at 50% to obtain brighter color images. Analyzed peptide intensities were within the linear range, thus allowing for a semiquantitative approach. Furthermore, ionization biases can be ruled out since regional-specific peptide formation was confirmed using microdissected cortical and medullary biopsies. Careful attention to detail and consistency throughout the experimental process avoided analyte migration during incubation. This is documented by the clear differentiation of medullary and cortical MS signals. Indeed, previous reports confirm that washes in organic or aqueous solutions did not cause migration of peptides across tissue sections (23, 43).

Ang III-generating enzyme activity was easily detected by MALDI imaging in the inner medullary region of the kidney. Specificity was verified via use of GluP, a specific APA inhibitor. These results are in contrast to earlier reports that showed higher levels of APA in renal cortex than in medulla (29, 46, 47). The regional differences in APA activity may be related to the analytical approaches used for the detection of enzyme activity. Whereas our studies used the natural peptide as a precursor, previous studies tested β-naphthylamide derivatives as substrates with spectrophotometric detection. Additionally, soluble and membrane-bound forms of APA were found to be differentially active (15). The MALDI imaging approach visualized active APA, whereas immunological approaches are targeted toward the detection of overall protein located in a tissue.

It was shown previously that PCP and PEP are capable of converting Ang II to Ang-(1–7), albeit with lower catalytic efficiencies than ACE2 (30, 51, 55, 57). Our study with ACE2, PCP, and PEP inhibitors indicates that cortical Ang-(1–7) formation in the presence of low Ang II concentrations is due primarily to the actions of ACE2. However, with higher Ang II concentrations, non-ACE2-dependent mechanisms may contribute to Ang-(1–7) generation. Thus, one must entertain the idea of PCP/PEP-mediated formation of Ang-(1–7), which is dependent on the local cellular milieu. This finding might be of particular interest for studies using chronic infusion of Ang II (1,000 ng·kg⁻¹·min⁻¹ for 4 wk), an established model of hypertension (26).

In conclusion, this study describes a new method for molecular imaging of the renal RAS using MALDI mass spectrometry. It allows for the simultaneous localization of Ang II metabolites and corresponding enzyme activities in kidney regions. Our approach allows for the use of natural substrates and characterization of multiple products. Herein we document the benefit of a MALDI-MS technique that combines specificity and selectivity for multiple analytes of interest with their spatial distribution on tissue sections and correlation to enzyme activity. Further studies applying this technique will elucidate the relevance for the role and site of Ang II processing in establishing RAS balance and cardiovascular and renal health. There are also plans to expand this tissue-imaging technique to Ang I, the physiologically most relevant precursor of Ang II, other tissues, and enzyme systems, as well as drug testing and development.

ACKNOWLEDGMENTS

We thank Drs. Richard Caprioli and Erin Seeley, Vanderbilt University, Nashville, TN, for training and analytical support in developing the MALDI imaging method. We acknowledge William C. Grunwald, Jr., Mary Key, and Naima S. Rodwan for excellent technical assistance. We give special thanks to our colleague Nathan M. Weir for invaluable support. We thank Dr. Robert C. Speth, Nova Southeastern University, Fort Lauderdale, FL, for providing glutamate phosphonate and Dr. Randall A. Skidgel, University of Illinois, Chicago, IL, for thoughtful discussion.

GRANTS

This work was supported by National Institutes of Health Grant R01-HL-093567 and fellowship 1-F32-DK-093226-01.

DISCLOSURES

No competing interests, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

N.G., K.M.E., D.R.C., and M.M. did the conception and design of the experiments; N.G. prepared the figures; N.G. and M.M. drafted the manuscript; N.G., K.M.E., D.R.C., and M.M. approved the final version of the manuscript.

REFERENCES


15. Greis KD. Innovative Methodology MALDI IMAGING OF RENAL ANGIOTENSIN METABOLISM E1023


