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Review Article

The Fundamentals for Studying Local Renin-Angiotensin System: Experience from Multi-omics Studies on Atherosclerosis

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Abstract

RAS is a complex bioactive system that comprises different pathways involved in the conversion of angiotensinogen into diverse bioactive peptides that exert various cellular effects through selective receptors. The specific combination of peptides and receptors defines the final response of a tissue to the system. RAS is involved in numerous pathologic mechanisms such as cellular growth, proliferation, differentiation, migration and apoptosis, as well as extra cellular matrix (ECM) remodeling and inflammation. We recently proposed an extended RAS system at the local tissue level that could be differentially regulated under various biological conditions in different organs. The expression of RAS components have been demonstrated in the arterial wall and during atherosclerotic lesion development. Despite the complexity of the system, most studies have focused on studying the functional aspects of certain axes of RAS at the tissue level in specific diseases. This review will discuss the aspects by which RAS should be studied and interpreted in terms of its biology at the physiological and pathophysiological levels, while focusing on the local differential expression and activity of RAS during atherosclerotic lesion development to provide a concrete platform for future studies.

Keywords: *Renin-Angiotensin System; Tissue; Systems Biology; Multi-Omics; Atherosclerosis*

Introduction

From a classical perspective, RAS was considered an endocrine system that starts with the cleavage of angiotensinogen, mainly produced by the liver, by the rate limiting enzyme renin expressed in the kidneys, to produce the decapeptide Angiotensin-I (Ang-I) [1] (Figure 1). The latter is then cleaved by the angiotensin converting enzyme (ACE) to produce the bioactive octapeptide Ang-II, which binds to its angiotensin type 1 receptor (AT1R) to exert its vasoconstrictive effects. However, this view was extended with the discovery of alternative enzymes to ACE, such as chymase, but also with the revelation of multiple bioactive angiotensin peptides, mainly Ang-(1-7), which can be produced by multiple alternative pathways and can bind to different receptors [1,2] (Figure 1). The system was further complexified with the identification of local paracrine RAS in different tissues, independent of the systemic endocrine system, including in the brain, kidneys, heart, ovary, pancreas and the vascular wall [1]. As a key player in multiple biological processes such as cellular growth, proliferation, differentiation, migration, and apoptosis, alterations in RAS expression have been implicated in multiple diseases including atherosclerosis, cardiac hypertrophy, type 2 diabetes, and renal fibrosis [1,3,4].

Most studies on tissue RAS focus on the local production of peptides in a specific tissue, or on the response to exogenous peptides treatment. However, few studies have simultaneously investigated both the local production and the response levels. In fact, both levels are interdependent, and studying each on its own will lead to inconclusive results about the actual effects of the system on the specific tissue under study. For instance, the local production of a bioactive molecule in a specific tissue does not necessitate that this molecule will exert its effects in that tissue unless the corresponding effectors (e.g. receptors) are locally present. Indeed, a local favorable environment should be available for a molecule to effectively exert its effects. This environment could be characterized by the expression of the

receptors of the molecule and the molecular pathways that can transduce the signal from the receptor to the final effectors, in addition to the absence of antagonizing pathways that may inhibit the action of this molecule. In fact, this should be the basis for interpreting the local effects of RAS at the tissue level. Indeed, all bioactive peptides and molecules of RAS rely on the presence of their corresponding receptors to exert their effects in a specific tissue, as well as on the local activities of synergistic and antagonistic pathways. Similarly, an expressed receptor cannot exert any effects without being bound and activated by the corresponding ligand. In addition, each peptide or molecule can bind different receptors and vice versa, which can lead to different, even opposite effects. The issue is even more complex in RAS because the same peptide may exert opposite effects depending on the receptor it activates, whereas the same receptor can exert similar effects despite binding different peptides. Therefore, the local effect of RAS depends on the combination of locally produced metabolites and their corresponding receptors. Indeed, one should consider both signal generation (enzymes) and signal response (receptors) when studying RAS and reporting its local effects in a tissue. Furthermore, the autocrine and paracrine effects of RAS should also be considered for a more complete view of the system at the tissue level [1,4].

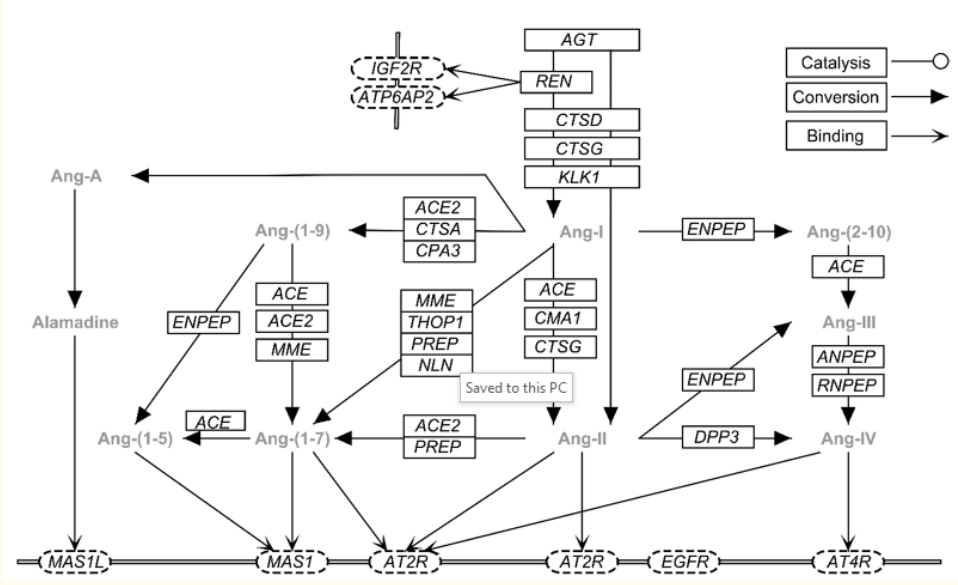

Figure 1: *The Renin-Angiotensin System (RAS). The metabolic cascades of angiotensin peptides are represented using gene symbols the enzymes and the receptors involved in the pathway. Angiotensin peptides are represented in grey using their usual abbreviation. Ang: Angiotensin; ACE: Angiotensin I Converting Enzyme; ACE2: Angiotensin I Converting Enzyme Type 2; AGTR1: Angiotensin II Type 1 Receptor; AGTR2: Angiotensin II Type 2 Receptor; ANPEP: Alanyl-Aminopeptidase; ATP6AP2: Prorenin/Renin Receptor; CMA1: Chymase 1; CPA3: Carboxypeptidase A3; CTSA: Cathepsin A; CTSD: Cathepsin D; CTSG: Cathepsin G; DPP3: Dipeptidyl-Peptidase 3; ENPEP: Glutamyl Aminopeptidase (Aminopeptidase A); IGF2R: Insulin-Like Growth Factor 2 Receptor; KLK1: Tissue Kallikrein; LNPEP: Leucyl/Cystinylaminopeptidase; MAS1: MAS1 Proto-Oncogene; MAS1L: Mas-Related G-Protein Coupled Receptor; MME: Membrane Metallo-Endopeptidase; MR: Mineralocorticoid Receptor; NLN: Neurolysin (Metallopeptidase M3 Family); PREP: Prolylendopeptidase; REN: Renin; RNPEP: Arginyl Aminopeptidase (Aminopeptidase B); THOP1: Thimetoligopeptidase 1.*

We have been studying the implications of RAS in atherosclerotic lesion development during the last decade by measuring the expression of multiple RAS components [2,5-8]. Based on the literature and on our studies, we have joined the different pathways of RAS, with their component enzymes and receptors, into one system including classical and newly discovered enzymes and receptors (Figure 1). We aimed to identify the organization of RAS in the atherosclerotic lesion at the mRNA, protein and metabolic levels using data obtained by our team, in addition to public microarray data sets available on the GEO database.

RAS expression in atherosclerosis

Our studies have shown the presence of similar transcriptional patterns of RAS in advanced atherosclerotic lesions and nearby macroscopically intact tissues (MIT), which were different from that in normal vascular tissue in both mouse and human [9]. This indicates that the organization of RAS might be established at early stages of atherosclerotic lesions development, which could be involved in lesion initiation and progression.

Similar to the atlas of tissue RAS, we have created a specific transcriptional map of RAS in atherosclerosis [2,9]. The RAS map in atheroma indicates that a highly expressed AGT could fuel the production of all downstream angiotensin peptides by locally expressed angiotensin metabolizing enzymes. However, the map also suggests that only Ang-II and Ang-IV could exert some effects by binding to their expressed receptors; whereas Ang-(1-7) may not be active, despite its possible production by the available enzymes, due to the very low expression levels of its corresponding receptors Mas1 and AGTR2 [9]. In fact, one can't give a global conclusion on RAS without considering the expression and activity of its mutual interactor, the corticosteroid system. Indeed, not only aldosterone is considered a major downstream effector of RAS, it has been also shown that RAS mutually interact with and activate both the mineralocorticoid and glucocorticoid activity [2,3,6,8]. Despite the high levels of the mineralocorticoid (MR) and the corticosteroid (GR) receptors, their effects might be limited by the low production of their ligands, aldosterone and cortisol, as suggested by the very low levels of both the aldosterone synthase (CYP11B2) and the cortisol synthase (CYP11B1) transcripts. However, one can't exclude that aldosterone could be imported from the circulation, and that it can exert its effects by binding to MR that is mainly expressed on epithelial cells facing the lumen of the vessel. Despite the modest correlation between the angiotensin and the corticosteroid systems at the enzymatic level, a strong correlation existed between the two systems at the receptor levels. Thus, it seems that while the signal generation of the two systems is independently regulated in atheroma, they are tightly correlated at the signal response level, which can lead to stronger synergy and stronger effects at the local tissue level.

Despite the fact that mRNA expression does not provide any support for functional relevance of the system, it may be an evidence for the local expression of the different components in the normal vessel wall and in atherosclerotic lesions.

Metabolic pathways of RAS in atherosclerosis

Further support for the transcriptomic data were obtained at the protein and metabolic levels. Proteomics analysis showed a very high peak in the upregulation of AGT at the protein level in advanced atherosclerotic plaques, compared with nearby MIT, which further confirms that a huge AGT repository locally fuels the production of all downstream angiotensin peptides in atheroma [10]. Unfortunately, none of RAS enzymes and receptors were differentially expressed at the protein level, which could be related to the mRNA expression that showed similar expression patterns between early and advanced lesions. In addition, this could be related to methodological issues as most of the receptors may have not been detected using the protein extraction method employed in this study.

The level of each angiotensin peptide is defined by its rate of production and degradation. Therefore, we constructed a model of angiotensin peptides production in atheroma, which allowed to reveal the *ex vivo* kinetics of bioactive angiotensin peptides production in advanced atherosclerotic lesions and nearby vascular MIT [7]. Our results showed that both tissue types can produce the different bioactive angiotensin peptides, including Ang-II, Ang-(1-7), Ang-III and Ang-IV; however, at different rates. The levels of Ang-II were higher in advanced lesions compared with MIT, which is in accordance with previous results showing increased Ang-II production in atheroma [11,12]. Based on the previously measured IC_{50} of the AT1R and AT2R to Ang-II, the measured concentrations of Ang-II produced by MIT and advanced lesions indicate that the AT2R is mainly activated in MIT, while both AT1R and AT2R could be activated in advanced lesions. Nevertheless, the transcriptomic profiles of the receptors in both human and mouse atherosclerotic lesions indicate that only the AT1R is expressed in atheroma [9], while the AT2R is not present, which favors the proatherogenic effects by the Ang-II peptide in advanced lesions.

Interestingly, our results confirm the local production of Ang-(1-7) as a major angiotensin peptide produced in both MIT and advanced lesions, which demonstrated high and effective concentrations that could induce its effects mainly through the $MasR^{7}$. Although no clear evidence is available about the protein expression of MasR in the vascular wall, studies investigating its effects indicate that MasR might

be present and active in atherosclerosis [13-15]. The increase of Ang-(1-7) concentration with time implies that its rate of production is higher than its rate of degradation in both MIT and advanced lesions, which is in line with its measured levels in both tissue types that are lower than its binding affinity to ACE, which cleaves Ang-(1-7) to Ang- (1-5) [16] (Figure 1).

The absence of Ang-III and the presence of Ang-IV may explain the decrease in Ang-II concentrations with time, by which Ang-IV can be directly produced through Ang-II cleavage. Nevertheless, the produced levels of Ang-IV seem to be biologically insignificant, being lower than the IC50 of Ang-VI binding to all receptors, including AT1R, AT2R and AT4R [17,18].

The cellular distribution of RAS in atherosclerosis

The 3 main cells that are involved in atherosclerotic lesion development are ECs, VSMCs and macrophages [19]. We have demonstrated in a previous review that these 3 cell types are likely to participate in angiotensin cleavage and bioactive peptides generation, but are also likely to differentially respond to angiotensin peptides through specific receptors on their surface [3] (Figure 2). Interestingly, our model indicates that VSMCs, the major drivers of atheroma development and progression, are the only cells that could be implicated in the conversion of AGT to Ang-I, which is considered the most proximal and rate-limiting step in renin-dependent angiotensin metabolism [1] (Figure 2). While the 3 cell types could participate in the generation of Ang-II and Ang-(1-7) in atheroma, further studies are required to elucidate the differential expression of AT1R, AT2R and MasR in these cell types during atheroma progression.

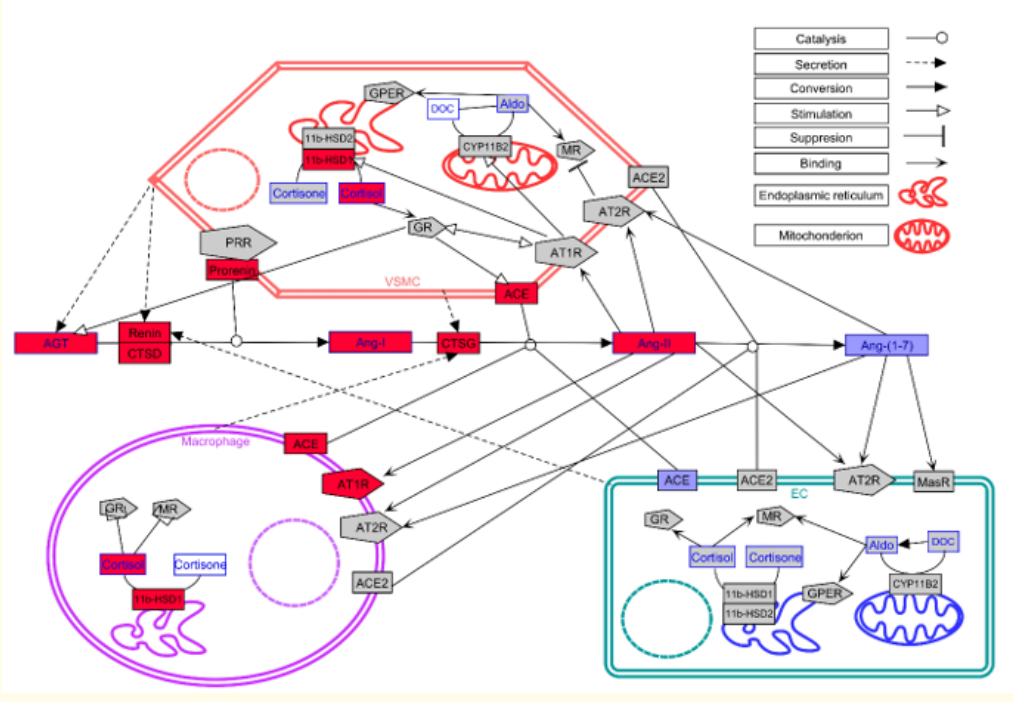

Figure 2: *Cellular distribution of extRAAS components in atherosclerotic lesions. Based on the expressed components, each cell contributes to a certain extent in extRAAS metabolites production. On the other hand, the produced metabolites would exert cell-specific effects based on the expressed receptors on each cell. Thus, the combination of the metabolites and receptors would define the final outcome on a particular cell type. Metabolites are labeled in blue. RAS components with increased, decreased or unknown expression in advanced atherosclerotic, compared to early lesions, are colored in red, blue and gray, respectively. 11b-HSD1: 11β-Hydroxysteroid Dehydrogenase Isoform 1; 11b-HSD2: 11β-Hydroxysteroid Dehydrogenase Isoform 2; Aldo: Aldosterone; AT1R: Angiotensin Type 1 Receptor; AT2R: Angiotensin Type 2 Receptor; DOC: 11-Deoxycorticosterone; EC: Endothelial Cell; GR: Glucocorticoid Receptor; GPER: G Protein-Coupled Estrogen Receptor 1; MR: Mineralocorticoid Receptor; VSMC: Vascular Smooth Muscle Cell; PRR: (Pro)Renin Receptor.*

Despite that all bioactive angiotensin peptides could be produced in the arterial wall and are altered during atherogenesis as a result of cell-specific differential expression, the same peptide may still exert different cell-specific effects depending on the combination of receptors available on the cell surface. Therefore, the expression pattern of RAS components, both at the signal generation and at the signal response levels should be further investigated in a cell-specific manner to obtain a clearer image of the system in atheroma. This will provide a more stringent basis for finding the most specific and efficient ways for the RAS-based treatment of atherosclerosis.

Tissue-specificity of RAS expression in atheroma

By comparing the expression profile of RAS obtained from atheroma to those obtained in other 23 different normal human tissues, it was clear that RAS possesses a tissue-specific co-expression pattern in atheroma [9]. The co-expression pattern constitute of two levels: the expression and the coordination levels. While the expression pattern provide an indication on the locally favored RAS pathways in a specific tissue, the coordination pattern informs about the interaction between the different pathways and how the system is balanced at the tissue level. The importance of the tissue-specific co-expression pattern of RAS in atheroma relies in that atheroma possesses specific characteristics that could be manipulated to modulate the system's local activity in atheroma without affecting its activity in other normally functioning tissues. In addition, the reproducibility of this organization across multiple human datasets, which included more than 800 human atheroma samples from different arterial beds, independent of inter-individual variability further supports the association of this co-expression pattern in atherosclerotic mechanisms. Thus, this could provide an easier way for future pharmacological approaches as it will not rely on personalized treatments. Moreover, the similarity of the co-expression pattern in the lesions from both human and apoE-deficient mice suggests that this animal model can be used as a robust model for studying RAS *in vivo*.

Conclusion and Future Directions

The one-axis investigations on RAS have led to the huge knowledge we have today about the role of RAS in local tissue physiology and pathophysiology. However, these data should now be connected to generate a global view of RAS at the paracrine level using systems biology approaches. In addition, they should also be linked with the global organization of the system in the human body as a whole. This should be performed at the RNA, protein and metabolic levels, which will provide more elaborate information on how the organization of the system is altered under a specific pathophysiological condition *in vivo*. This will help reach more specific and efficient targeting of RAS in a disease-specific manner using the most efficient combination of therapeutics that target specific enzymes and receptors that can get the system back into its normal balanced state. In addition, understanding the mechanisms by which the system is altered and maintained during pathogenesis will provide the basis for the discovery of new therapeutics that can modulate the global organization of RAS rather than targeting one enzyme or one pathway.

Despite the comprehensive information provided by our studies, they do not provide the full image at the protein and the metabolic levels. In addition, further analysis should be done to include novel pathways of RAS including the Ang-(1-12) and the Angiotensin A/Alamandine/MrgD axes [1]. Indeed, the complexity of the system is increasing by the discovery of new peptides and pathways. Therefore, these molecules warrant further studies in order to elucidate their clinical importance and the way they participate in the final effects of RAS at the local tissue level.

Conflict of Interest Disclosure

The author declare that he has no conflict of interest to declare.

Bibliography

1. Nehme A., *et al*. "An Update on the Tissue Renin Angiotensin System and Its Role in Physiology and Pathology". *Journal of Cardiovascular Development and Disease* 6 (2019).

2. Nehme A., *et al*. "Atlas of tissue renin-angiotensin-aldosterone system in human: A transcriptomic meta-analysis". *Scientific Reports* 5 (2015): 10035.

3. Nehme A and Zibara K. "Cellular distribution and interaction between extended renin-angiotensin-aldosterone system pathways in atheroma". *Atherosclerosis* (2017).

4. Nehme A and Zibara K. "Efficiency and specificity of RAAS inhibitors in cardiovascular diseases: how to achieve better end-organ protection?" *Hypertension Research* (2017).

5. Hodroj W., *et al*. "Increased insulin-stimulated expression of arterial angiotensinogen and angiotensin type 1 receptor in patients with type 2 diabetes mellitus and atheroma". *Arteriosclerosis, Thrombosis, and Vascular Biology* 27 (2007): 525-531.

6. Ayari H., *et al*. "Mutual amplification of corticosteroids and angiotensin systems in human vascular smooth muscle cells and carotid atheroma". *Journal of Molecular Medicine* 92 (2014): 1201-1208.

7. Nehme A., *et al*. "The kinetics of angiotensin-I metabolism in human carotid atheroma: An emerging role for angiotensin (1-7)". *Vascular Pharmacology* (2016).

8. Bricca G., *et al*. "Local Angiotensin Pathways in Human Carotid Atheroma: Towards a Systems Biology Approach". *Conference Paper in Science* (2015).

9. Nehme A., *et al*. "Transcriptomic Analysis Reveals Novel Transcription Factors Associated With Renin-Angiotensin-Aldosterone System in Human Atheroma". *Hypertension* (2016).

10. Nehme A., *et al*. "Functional pathways associated with human carotid atheroma: a proteomics analysis". *Hypertension Research* (2019).

11. Ihara M., *et al*. "Increased chymase-dependent angiotensin II formation in human atherosclerotic aorta". *Hypertension* 33 (1999): 1399-1405.

12. Miyazaki M and Takai S. "Tissue angiotensin II generating system by angiotensin-converting enzyme and chymase". *Journal of Pharmacological Sciences* 100 (2006): 391-397.

13. Jawien J., *et al*. "Angiotensin-(1-7) receptor Mas agonist ameliorates progress of atherosclerosis in apoE-knockout mice". *Journal of Physiology and Pharmacology* 63 (2012): 77-85.

14. Hayashi N., *et al*. "The counter regulating role of ACE2 and ACE2-mediated angiotensin 1-7 signaling against angiotensin II stimulation in vascular cells". *Hypertension Research* 33 (2010): 1182-1185.

15. Alsaadon H., *et al*. "Increased aortic intimal proliferation due to MasR deletion in vitro". *International Journal of Experimental Pathology* 96 (2015): 183-187.

16. Chappell MC., *et al*. "Metabolism of angiotensin-(1-7) by angiotensin-converting enzyme". *Hypertension* 31 (1998): 362-367.

17. Bosnyak S., *et al*. "Relative affinity of angiotensin peptides and novel ligands at AT1 and AT2 receptors". *Clinical Science (London, England 1979)* 121 (2011): 297-303.

18. Handa RK. "Metabolism alters the selectivity of angiotensin-(1-7) receptor ligands for angiotensin receptors". *Journal of the American Society of Nephrology* 11 (2000): 1377-1386.

19. Nehme A. "Targeting Tissue-Specific Enzymatic Cascades of Local Angiotensin System in Human Atheroma". (2015).

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