Mako: NSU Undergraduate Student Journal

Volume 2020 *Spring* Article 2

February 2020

ACE2/Ang1-7 MAS AXIS: THE COUNTER-REGULATOR OF THE CLASSICAL RENIN ANGIOTENSIN SYSTEM

Widelyne Dorsainval
Nova Southeastern University, wd250@mynsu.nova.edu

ACE2/Ang1-7 MAS AXIS: THE COUNTER-REGULATOR OF THE CLASSICAL RENIN ANGIOTENSIN SYSTEM

ACE2/Ang1-7 MAS AXIS: THE COUNTER-REGULATOR OF THE CLASSICAL RENIN ANGIOTENSIN SYSTEM

Widelyne Dorsainval
Dr. Robert C. Speth (Faculty Advisor)
Nova Southeastern University BIOL4950
December 8, 2017

Correspondence:
Robert C. Speth, Ph.D., FAAAS
Department of Pharmaceutical Sciences
College of Pharmacy
Nova Southeastern University
Fort Lauderdale, FL 33328
Email: rs1251@nova.edu

ACE2/Ang1-7 MAS AXIS: THE COUNTER-REGULATOR OF THE CLASSICAL RENIN ANGIOTENSIN SYSTEM

Abstract

To maintain homeostasis, the renin-angiotensin system (RAS) works to regulate the cardiovascular, renal, respiratory, and neurological systems of the body. However, there is evidence that abundant amounts of certain components of the RAS have detrimental effects and enhance disease. Recent studies have proved that there is a lesser known RAS which acts to counterbalance the classical RAS. To better understand their interaction, the effects of the alternate RAS against the classical RAS in the homeostatic systems of the body are researched and analyzed. The classical pathway employs these components: angiotensin converting enzyme (ACE), angiotensin II (AngII), and angiotensin II type 1 receptor (AT1); the alternate pathway involves: angiotensin converting enzyme 2 (ACE-2), angiotensin 1-7 (Ang1-7), and a Mas receptor. There is growing affirmation that the novel alternate pathway RAS can play a significant role in the advancement and progression of treatment for cardiovascular, renal, neurological and respiratory diseases. Its components possess the potential to serve as templates for the development of new drugs to mirror or enhance their interactions with the classical RAS when necessary.

ACE2/Ang1-7 MAS AXIS: THE COUNTER-REGULATOR OF THE CLASSICAL RENIN ANGIOTENSIN SYSTEM

The purpose of science is to aim to explain and understand complex processes and interactions within nature, and discover new ones that can help humanity. Throughout the history of medical studies, there have been countless cases focused on unraveling the intricate steps involved in the human body's care for itself. One of the heavily studied processes is the renin-angiotensin system (RAS) which is a pivotal player in many homeostatic functions of the body.

The Classical Renin-Angiotensin System (RAS)

Angiotensinogen is the precursor of Angiotensin II (AngII) and is hydrolyzed by the enzyme renin to form Angiotensin I (AngI). AngII is formed when the two carboxyterminal amino acids of AngI are cleaved by angiotensin-converting enzyme (ACE). The RAS works to regulate functions in the heart, kidneys, lungs, and brain.

Upon binding to the Ang II type 1 receptor (AT1R), Ang II stimulates vasoconstriction and secretion of the steroid hormone, aldosterone, which mediates sodium reabsorption and water retention. The RAS plays an important role in regulating blood volume and systemic vascular resistance, which together influence cardiac output and arterial pressure. AngII, a major member in the RAS, has direct and indirect effects on the heart. AngII has been known to act as a growth factor in the heart. In isolated cardiac cells, angiotensin II induces hyperplasia

(fibroblasts) or hypertrophy (myocytes) (Danser, 1996). It also enhances myocardial contractility and metabolism. AngII directly regulates thirst and salt appetite via the central nervous system. Several studies have found that the level of water intake correlates with the AngII plasma levels; water intake is substantially reduced when the renin-angiotensin system is inhibited (Sica, 2001).

The RAS has its roots in the renal system as well. Renin in secreted from juxtaglomerular cells in the kidneys. Ang II reduces single nephron glomerular filtration rate, glomerular plasma flow and increases both afferent and efferent arteriolar resistance; it is basically one of the overall regulators of renal hemodynamics (Kobori, 2007). Ultimately, the renal RAS is involved in sodium balance, bodily fluid volume, and arterial blood pressure. The renal and cardiovascular systems affect each other because when cardiac output declines, decrease in pressure from reduced renal blood blow stimulates the baroreceptors of the kidney vessels, the juxtaglomerular cells, and renin secretion is enhanced (Kobori, 2007).

As far as the RAS presence in the lungs, at a cellular level, angiotensin and aldosterone activate oxidant stress signaling pathways that decrease levels of bioavailable nitric oxide, increase inflammation, and promote cell proliferation, migration, extracellular matrix remodeling, and fibrosis (Maron, 2014).

The Counter-regulatory Renin-Angiotensin System

The components of the alternate pathway involve angiotensin converting enzyme-2 (ACE-2), Angiotensin 1-7, and a Mas receptor. ACE-2, a homologue of ACE from the classical RAS, functions as a carboxypeptidase that inactivates Ang II and is a negative regulator of the system. The difference between ACE and ACE2 is that ACE2 cleaves only a single residue from Ang I to generate Ang1-9, and a residue from Ang II to generate Ang1-7. In this way, ACE2 negatively regulates the RAS by inactivating Ang II and/or competing with ACE for the

ACE2/Ang1-7 MAS AXIS: THE COUNTER-REGULATOR OF THE CLASSICAL RENIN ANGIOTENSIN SYSTEM

substrate Ang I. A study by Crackower et. al (2002) presents data confirming the function of ACE2 as a regulator of the RAS modulating endogenous levels of Ang I and Ang II in mouse hearts. Ang1-7, which possesses biological actions, is an angiotensin II peptide derivative where the last carboxy amino acid, phenylalanine, is deleted. In early studies, Ang1-7 was initially shown to mimic some effects of AngII including release of prostanoids and vasopressin. This is old and disregarded information because the methods used to reach this conclusion have been deemed incorrect. Recent studies show that Ang-(1-7) induces systemic and regional vasodilation, diuresis and natriuresis, and exerts antiproliferative and antigrowth effects in vascular smooth muscle cells, cardiac myocytes and fibroblasts as well as glomerular and proximal tubular cells (Ferrario, 2014). The Mas Protooncogene encodes a protein with seven hydrophobic transmembrane domains, considered to be an "orphan" G protein coupled receptor. In a study by Santos and colleagues (2003), radioligand binding with autoradiography on kidneys of Mas-deficient mice was conducted to demonstrate that the G protein coupled receptor, Mas, binds Ang1-7 and is involved in its biological actions. Without this receptor, the AngII derivative is essentially inactive. Knowing the role that each of these individuals take in the alternate axis is critical in understanding how the counter-regulation works.

The Brain Renin-Angiotensin System

There is substantial evidence that activation of the RAS in the brain is involved in water and sodium intake. Thirst and sodium depletion are monitored by osmoreceptors and/or sodium receptors located at the periphery and AT_1 receptors in the brain (Johnson, 2008). Additionally, the activities of the brain RAS are achieved by influencing the autonomic nervous system, the baroreflex sensitivity, and vasopressin (AVP) release (Speth et al., 2003). The CNS regulates blood pressure by integrating neurohormonal signals to alter intake (water and salt) and output

(renal excretion). In a way, the renin angiotensin system connects the cardiovascular, renal, and CNS systems to keep the body's homeostasis as leveled as possible. Besides knowledge on all of these incredible findings, new research has revealed that there is more than just the traditional renin-angiotensin pathway. The classical pathway employs these familiar components ACE, AngII, and AT1 receptor, however, the alternate pathway involves deviations of some of these and a different receptor. It is believed that the classical renin-angiotensin system is counterbalanced by a prominent ACE2/Ang1-7/Mas Receptor activation, which is being studied in depth to better understand how the body acts to maintain homeostasis between systems.

The Renin-Angiotensin System and Cardiovascular Disease

Many great discoveries have been made on the implications of the ACE2/Ang1-7/Mas axis in diseases involving the classical renin-angiotensin system including cardiovascular disease. Heart failure, a cumulative of previous conditions that weaken or stiffen the heart, affects approximately 5.7 million adults in the United States, and cardiovascular disease is the leading cause of death. Unraveling the involvement of the classical RAS has already made ways for significant improvements in treatments for heart diseases. However, the introduction of the countering system has provided more insight on how the body works to maintain its homeostasis. A study by Tyrankeiwicz and colleagues (2017) was done on the activation pattern of the ACE2/Ang1-7 and ACE/AngII pathways in course of heart failure. It was assessed by multiparametric MRI *in vivo* in Tgαq*44 mice. The authors explained that "Tgαq*44 mice are a transgenic model of slowly developing heart failure and peripheral endothelial dysfunction due to cardiomyocyte-specific overexpression of G protein αq*44 (Tyrankeiwicz , 2017). They analyzed systemic and local changes in ACE/ACE-2 balance in mice which were undergoing heart failure. The pattern of changes in the heart and in the aorta was compatible with activation

of ACE2 followed by activation of ACE. They found that the early stage of heart failure was associated with upregulation of ACE2/Ang1-7 and the end stage by not only downregulation of ACE2/Ang1-7 but also upregulation of the ACE/AngII pathway. ACE/ACE-2 balance seemed to determine the decompensation of heart failure in this model. Additionally, in isolated hearts from mRen2 (renin 2 tandem duplication of renin 1) transgenic rats, scientists conducting another study showed that chronic ACE2 inhibition with the drug MLN-4760 for 28 days worsened cardiac remodeling that was associated with increased cardiac Ang II levels (Ferrario, 2014). These outcomes further enforce that the RAS involves two counter-regulatory axes (ACE/AngII/AT_1R) and ACE2/Ang1-7/Mas, and their balance is fundamental in maintaining cardiovascular homeostasis. Hypertension is also one of the most common causes of cardiovascular disease that affects billions of individuals worldwide. Ang II, from the classical pathway, contributes to the regulation of blood pressure by influencing constriction of vascular smooth muscle cells and sodium and water volume homeostasis as well as aldosterone secretion (Ferrario, 2014). In an article by Castro and colleagues (2015), they studied the key role of ACE2 and the vasoactive peptide Ang1-7 as counter-regulators of the ACE/Ang II/AT_1R axis as well as the biological properties that allow them to regulate blood pressure (BP) and cardiovascular remodeling. They found that the ACE2/Ang-(1-7)/MasR axes has effects opposite to those of the ACE/Ang II/AT1R axis, such as decreased proliferation and cardiovascular remodeling, and increased production of NO and vasodilation (Castro, 2015). When investigating ACE2 from a genetic view point in hypertensive rats, Crackower and colleagues (2002) discovered that the Ace2 gene maps to a defined quantitative trait locus on the X chromosome. A quantitative trait locus is a section of DNA which correlates with variation in a phenotype and is usually linked to, or contains, the genes which control that phenotype. In all of

the hypertensive rat strains, ACE2 mRNA and protein expression were significantly reduced and AngII levels high (Crackower, 2002). ACE2 is such a key player in the counteraction of the classical system in this case because it metabolizes the vasoconstrictive, hypertrophic, proliferative angiotensin II into a more favorable angiotensin-(1-7) when necessary.

The Renin-Angiotensin System and Kidney Disease

Kidney disease is a major worldwide problem and patients with chronic kidney disease (CKD) are at increased cardiovascular risk. CKD is characterized by enhanced activity of the RAS which progresses to both cardiac and renal injury. Factors beyond the traditional RAS are involved in the progression of the disease. According to Burrel et. al (2004), ACE2 acts in a counter-regulatory manner to ACE, modulating the balance between vasoconstrictors and vasodilators within the heart and kidney, and playing a significant role in regulating cardio and renal function. Additional studies show that ACE2 has renoprotective effects because when ACE2 is deleted or inhibited, pathology ensues (Burrel, 2004). Cao et. al (2012), investigated heart and kidneys in mice that were diseased (induced by subtotal nephrectomy where they cut out most but not all of the nephrons in the kidneys). The scientists found that acute renal mass reduction with nephron removal led to reduction in renal ACE2 activity, but marked increase in cardiac ACE2 activity. This is one factor that enforces the tie between renal and cardiac health. As ACE2 production is depleted because of the lack of nephrons, the heart is releasing more ACE2 to compensate. Since the nephrons are the functional units of the kidney that handle excreting excess water concentration in the blood, their absence means an increase in blood volume and therefore blood pressure. Vasodilation reduces vascular resistance to blood flow and therefore lowers blood pressure. This may be why the heart increases its ACE2 activity which is known to have dilatory functions. Although quite intriguing results, it is unknown if these

conditions persist long term. The authors suggest that adjunctive therapies to ACE inhibition, such as strategies that lead to increases in ACE2 activity over and above levels seen in normal physiology, may be needed in order to combat the cardio-renal complications of chronic kidney disease.

The Renin-Angiotensin System and Lung Disease

Pulmonary fibrosis is a progressive and fatal lung disease characterized by chronic inflammation, the migration and proliferation of fibroblasts, the accumulation of the extracellular matrix (ECM), and remodeling of the lung parenchyma (functional tissue). Currently, there are no effective antifibrotic therapies for pulmonary fibrosis. As expressed in an article by Meng et.al (2013), a growing body of evidence indicates that AngII plays a key role in the initiation and the maintenance of lung fibrosis, in other words, it negatively effects lung recovery by enhancing fibrosis, increasing inflammation and other factors. The recent discovery of the ACE2/Ang-(1-7)/Mas axis offers an alternative approach for counter-regulating the ACE/AngII/AT1R axis to produce more beneficial effects. Ang(1-7) has been shown to counteract the detrimental effects of AngII in the lungs (Meng, 2013). The ACE2/Ang-(1-7)/Mas axis may potentially offer a novel therapeutic strategy for pulmonary fibrosis. However, the exact molecular mechanism by which the ACE2/Ang-(1-7)/Mas axis protects against pulmonary fibrosis remains unclear. Affirmation of a beneficial role of ACE-2/ANG1-7 is strengthened by in vivo studies of animals that utilized genetic manipulation of ACE-2 or specific inhibitors of ACE-2 to establish a protective role of the enzyme (Uhal, 2014). Previous work by Uhal et al. showed that ACE-2 is protective against experimental fibrosis, but is down-regulated in both human lung fibrosis and experimental lung fibrosis in animal models. More recently, the same researchers analyzed lung biopsy specimens from humans and rats with fibrosis and

demonstrated that the mRNA transcript for ACE2 was producing low levels of protein product which in-turn meant decreased levels of enzymatic activity. Additional findings revealed that administration of experimental Ang1-7 and ACE2 reduced collagen deposition, decreased AT1 receptor protein levels in the rat lungs, and that protective effects against lung fibrosis were also obtained by overexpression of ACE-2 (Uhal, 2014).

Novel Aspects of the Brain Renin-Angiotensin System and Neurodegenerative Disease

Besides its control of systemic blood pressure, it has been clarified that the local brain RAS plays an important role in a variety of neuronal functions. Recent clinical trials have revealed that administration of AT_1 Angiotensin receptor blockers (ARBs) not only lowered blood pressure but only prevented the onset of stroke (Mogi, 2011). Further studies have shown that the inhibition of different components of the classical RAS, specifically ACE and the AT_1 receptor, has neuroprotective effects on the brain and prevents other detrimental events like epilepsy (Becari, 2010). Similarly, stimulation of the AT_2 receptor brings neuronal regeneration after injury and the inhibition of pathological progression. Ang-(1–7) mediates its antihypertensive effects by stimulating the synthesis and release of vasodilator prostaglandins and nitric oxide (Albrecht, 2006). In another recent study by Mecca and associates (2011), central administration of ANG-(1–7) reduced neurological deficits and infarct size in a rat model of ischemic stroke, demonstrating evidence of the special cerebroprotective properties of this peptide.

The Counter-regulatory Renin-Angiotensin System: Novel Therapeutic Approaches

The newly uncovered ACE2/Ang-(1-7)/Mas axis provides possibility for new treatments for RAS related diseases like pulmonary fibrosis, hypertension, heart failure, and kidney disease. Many great treatments have already been devised to suppress the classical system which is

beneficial because they give way for the alternate system to better perform. However, drugs to directly interact with the new system are on the rise. The counter-regulatory axes of renin angiotensin system play an important role in the cardiovascular repair. A study by Singh et. al (2015) suggests that ACE2/Ang-(1-7)/Mas pathway stimulates functions of CD34+ (a common cell-surface marker) cells that are reparative and regenerative of the cardiovascular framework. New evidence has shown that ACE2 and Mas receptor are actually expressed in these cells which are vasoprotective (Singh, 2015). These findings imply that pharmaceutical activation of ACE2/Ang-(1-7)/Mas axis is a promising approach for enhancing the reparative outcomes present in cell-based therapies. Knowing the benefits that lie in the counterregulatory powers of the alternate system against the classical system, Patel and colleagues (2016) introduced a well-studied tool to enhance ACE2 action: recombinant human ACE2 (rhACE2). In a randomized, placebo-controlled study, these scientists administered intravenous rhACE2 to healthy human subjects and found that it was well-tolerated. RhACE2 provided beneficial effects against Ang II-induced heart failure. Patients that had suppressed Ang-(1-7) and rising Ang II amounts experienced worsened HF symptoms and longer hospitalization. Whereas once the rhACE2 was given, Ang1-7 levels increased and AngII leveled (Patel, 2016). Thus, using rhACE2 as a therapy is very much a viable option. Several ACE2 activators and Ang 1–7/MasR agonists have been developed. Also, gene therapy approaches could be utilized to achieve a more tissue-specific delivery of ACE2/Ang 1–7. The stimulation of the ACE2/Ang-(1–7)/Mas axis has been successfully used to prevent and reverse pulmonary hypertension and fibrosis in animals. ACE2 activation or induction of ACE2 overexpression by gene transfer efficiently prevented and, more importantly, reversed the increase of right systolic ventricular pressure (RSVP), pulmonary fibrosis, and imbalance of the RAS (Patel, 2016). Novel approaches, including oral ACE2 and

Ang 1–7 biencapsulated in plant cells, have been designed and used in preclinical studies, showing promising antihypertensive, anti-inflammatory and cardioprotective effects. This is one of the most significant findings recently because a system to generate human ACE2 and Ang-(1-7) within plant chloroplasts using transplastomic technology has been invented. The native human ACE2 cDNA and synthetic Ang-(1-7) DNA sequences were cloned into a chloroplast transformation vector, and for efficient delivery of the proteins into circulation, a carrier protein, cholera nontoxic B subunit (CTB), was fused to the N terminal of both therapeutic proteins (Shenoy, 2014). Doing this facilitates their transmucosal delivery by binding to certain receptors (GM1) present on the intestinal epithelial cells. Additional modifications were made between CTB and the therapeutic proteins to eliminate steric hindrance and aid systemic release of the therapeutic proteins after they have entered the system via ligand–receptor complex formation on the surface of epithelial cells (Shenoy, 2014). This approach by Shenoy and colleagues is remarkable because it develops a system of a plant-based oral delivery which will overcome challenges like repetitive intravenous dosing, cost of manufacturing protein stability, and patient compliance. It is the next step in being able to exploit the alternate system to reach its fullest beneficial potential. This study provides proof that the creation of a low-cost plant based oral delivery system for ACE2 or Ang-(1-7) bioencapsulated in plant chloroplasts is most certainly possible (Shenoy, 2014).

Conclusions and Future Directions

The complexity of the RAS is far beyond what was suspected years ago and there are still many unanswered questions about the novel RAS pathway. Learning about this counteracting pathway gives more insight on how the body is equipped to keep its equilibrium. There is growing evidence that the novel components of the RAS [Ang-(1–7), ACE2, and Mas] may be a

ACE2/Ang1-7 MAS AXIS: THE COUNTER-REGULATOR OF THE CLASSICAL RENIN ANGIOTENSIN SYSTEM

significant piece to the advancement and progression of treatment for cardiovascular, kidney, neurological and respiratory diseases. Additionally, because of their counterregulatory actions, they serve as templates for the development of new drugs to mirror or enhance their interactions with the classical renin angiotensin system when necessary.

References

Ahmad, S., Nagata, S., Varagic, J., Ferrario, C. M. (2014). ACE2: Angiotensin II/Angiotensin-(1-7) balance in cardiorenal injury. Current Hypertension Reports, 16(3), 420th ser. doi:https://doi.org/10.1007/s11906-014-0420-5

Albrecht, D., O. von Bohlen und Halbach, O. (2006). The CNS renin-angiotensin system. Cell and Tissue Research, 326(2), 599-616. doi:https://doi.org/10.1007/s00441-006-0190-8

Becari, C., Salgado, M., Oliveira, J., Garcia-Cairasco, N., Costa-Neto, C., Pereira, M. (2010). Inhibition of the renin-angiotensin system prevents seizures in a rat model of epilepsy. Clinical Science, 119(11), 477-482. doi:10.1042/CS20100053

Burrell, L. M., Johnston, C. I., Cooper, M., Tikellis, C. (2004). ACE2, a new regulator of the renin-angiotensin system. Trends in endocrinology and metabolism: TEM, 15(4), 166-169. doi:10.1016/j.tem.2004.03.001

Castro, P. F., Oyarzún, A., Mondaca-Ruff, D., Azocar, A., Mendoza-Torres, E., Lavandero, S. (2015). ACE2 and vasoactive peptides: novel players in cardiovascular/renal remodeling and hypertension . Therapeutic Advances in Cardiovascular Disease, 9(4), 217-237. doi:10.1007/s11906-014-0420-5

Chrysant, Steven. G. (2005). Possible pathophysiologic mechanisms supporting the superior stroke protection of angiotensin receptor blockers compared to angiotensin-converting enzyme inhibitors: clinical and experimental evidence. *Journal of Human Hypertension, 19*, 923-931. doi:10.1038/sj.jhh.1001916

Crackower, M. A., Sarao, R., Oudit, G. Y., Yagil, C., Backx, P. H., Ferrario, C. M. (2002). Angiotensin-converting enzyme 2 is an essential regulator of heart function. Nature, 417(6891), 822-828.

Ferrario, C. M., Chappell, M. C., Tallant, E. A., Brosnihan, K. B., Diz, D. I. (1997). Counterregulatory Actions of Angiotensin-(1-7) [Abstract]. *Hypertension, 30*, 535-541. doi:https://doi.org/10.1161/01.HYP.30.3.535

Ferreira, A. J., Murc ̧a, T. M., Fraga-Silva, R. A., Castro, C. H., Santos, R. A., Raizada, R. K. (2012). New Cardiovascular and Pulmonary Therapeutic Strategies Based on the Angiotensin-Converting Enzyme 2/Angiotensin-(1–7)/Mas Receptor Axis. *International Journal of Hypertension, 2012*, 13. doi:10.1155/2012/147825

Gopallawa, I., Uhal, B. D. (2014). Molecular and cellular mechanisms of the inhibitory effects of ACE-2/ANG1-7/Mas axis on lung injury. *Curr Top Pharmacol., 18*(1), 71-80.

ACE2/Ang1-7 MAS AXIS: THE COUNTER-REGULATOR OF THE CLASSICAL RENIN ANGIOTENSIN SYSTEM

Gulman, J., Blume, A., Unger, T., Gohlke, P. (2002). The renin-angiotensin system in the brain: possible therapeutic implications for AT1- receptor blockers. *Journal of Human Hypertension,16*, 64-70. Retrieved from https://www.nature.com/jhh/journal/v16/n3s/pdf/1001442a.pdf?origin=ppub.

Hamming, I., Cooper, M., Haagmans, B., Hooper, N., Korstanje, R., Osterhaus, A., Timens, W., Turner, A., Navis, G.,van Goor, H. (2007), The emerging role of ACE2 in physiology and disease. J. Pathol., 212: 1–11. doi:10.1002/path.2162

Horiuchi, M., Mogi, M. (2011), Role of angiotensin II receptor subtype activation in cognitive function and ischaemic brain damage. British Journal of Pharmacology, 163(6): 1122–1130. doi:10.1111/j.1476-5381.2010.01167.x

Jan Danser, A. H., Schalekamp, A. (1996). Is there an internal cardiac renin-angiotensin system. *Heart*, 76, 28-32

Kobori, H., Nishiyama, A., Nangaku, M. L., Navar, G. (2007). The Intrarenal Renin-Angiotensin System: From Physiology to the Pathobiology of Hypertension and Kidney Diseases. *Pharmacological Reviews, 59*(3), 251-287. doi:DOI: 10.1124/pr.59.3.3

Maron, B. A., Leopold, J. A. (2014). The role of the renin-angiotensin-aldosterone system in the pathobiology of pulmonary arterial hypertension (2013 Grover Conference series). *Pulmonary Circulation*, *4*(2), 200–210. http://doi.org/10.1086/675984

Meng, Y., Li, W., Li, T., Luo, W., Li, X., Huang, S. (2013). Angiotensin-Converting Enzyme 2/Angiotensin-(1-7)/Mas Axis Protects against Lung Fibrosis by Inhibiting the MAPK/NF-κB Pathway. American Journal of Respiratory Cell and Molecular Biology, 50(4).

Patel, V. B., Zhong, J. C., Grant, M. B., Audit, G. Y. (2016). Role of the ACE2/Angiotensin 1–7 axis of the Renin-Angiotensin System in Heart Failure. *118*(8), 1313-1326. doi: 10.1161/CIRCRESAHA.116.307708

Santos, R. A. S., Silva, A. C. S. E., Maric, C., Silva, D. M. R., Machado, R. P., de Buhr, I., . . . Walther, T. (2003). Angiotensin-(1-7) is an endogenous ligand for the G protein-coupled receptor Mas. *Proc Natl Acad Sci U S A, 100*(14), 8258-8263. doi:DOI 10.1073/pnas.1432869100

Shenoy, V., Kwon, K.-C., Rathinasabapathy, A., Lin, S., Jin, G., Song, C., Raizada, M. K. (2014). Oral Delivery of Angiotensin-Converting Enzyme 2 and Angiotensin-(1-7) Bioencapsulated in Plant Cells Attenuates Pulmonary Hypertension. *Hypertension*, *64*(6), 1248–1259.

Sica, D. A. (2001). Angiotensin II and thirst: therapeutic considerations. *Conjestive Heart Failure*, 7(6), 325-328.doi:10.1111/j.1527-5299.2001.00274.x

Singh, N., Joshi, S., Guo, L., Baker, M. B., Y. L., Castellano, R. K. (2015). ACE2/Ang-(1-7)/Mas axis stimulates vascular repair-relevant functions of CD34 cells. *Am J Physiology Heart Circ Physiology*, *309*(10), 1697-1707. doi: 10.1152/ajpheart.00854.2014

Speth RC, Brown TE, Barnes RD, Wright JW. 2003. Brain angiotensinergic activity: the state of our current knowledge. *Proc.West Pharmacol.Soc* 46:11-5

Sumners, C., Mecca, A., Regenhardt, R., O'Connor, T., Joseph, J., Katovich, M. (2011). Cerebroprotection by angiotensin-(1-7) in endothelin-1-induced ischaemic stroke. Experimental Physiology, 96(10), 1084-1096. doi:10.1113/expphysiol.2011.058578

Tyrankiewicz, U., Olkowicz, M., Jablonska, M., Orzyłowska, A., Bar, A., Chlopicki, S. (2017). Activation pattern of ACE2/Ang-(1-7) and ACE/Ang II pathway in course of heart failure

ACE2/Ang1-7 MAS AXIS: THE COUNTER-REGULATOR OF THE CLASSICAL RENIN ANGIOTENSIN SYSTEM

assessed by multiparametric MRI in vivo in Tgαq*44 mice. *Journal of Applied Physiology*. doi:10.1152/japplphysiol.00571.2017.

Xu, P., Lazartigues, E., Sriramula,, S. (2011). ACE2/ANG-(1–7)/Mas pathway in the brain: the axis of good. *American Physiological Society*, *300*(4). doi: 10.1152/ajpregu.00222.2010