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Review Article

ACE 2/Ang (1-7)/Mas, Non-conventional RAS Axis: Endogenous Contributor of Cardio, and Reno-protective Responses

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Abstract

The Renin angiotensin system (RAS) is an intricate pathway that regulates homeostasis. The proliferative arm of RAS that includes angiotensin converting enzyme (ACE), angiotensin II (Ang II), and angiotensin II type 1 receptor (AT1R) contributes to pathophysiological responses such as vasoconstriction, hypertension, and cardiovascular, cerebral, and renal complications. The discovery of angiotensin converting enzyme 2 (ACE 2), the analog of ACE, synthesizes angiotensin (1-7) [Ang (1-7)] from Ang II or even from angiotensin I (Ang I) or angiotensin (1-9) [Ang (1-9)] less efficiently. Ang (1-7) is a biologically active peptide, also the endogenous antagonist of Ang II, and opposes the derogatory activities of the conventional axis of RAS. The functional binding site for Ang (1-7) is a G protein-coupled, AT1R and Mas receptor. ACE 2/Ang (1-7)/Mas is therefore termed the non-conventional axis of RAS, which is an endogenous counter-regulatory arm within RAS against the conventional axis. In conclusion, the ACE2/Ang (1-7)/Mas axis is the protective component of the renin-angiotensin system, counteracting the effects of angiotensin II and supporting cardiovascular and renal health. However, the detailed molecular mechanism of RAS as a whole still remains elusive due to the fact that RAS is highly organ, gender, and species/strain-specific, and much focus is needed in this area.

This review examines the non-conventional RAS axis, focusing on its impact on cardiovascular and renal systems, metabolism, and its potential interactions with AT1R, angiotensin II type 1 receptor (AT2R), and Mas. Evidence suggests that enhancing the ACE 2/Ang (1-7)/Mas axis can improve outcomes in diseases related to RAS dysregulation, highlighting its promise as a therapeutic target for cardiovascular and renal conditions. Additionally, the review explores how leveraging the ACE 2/Ang (1-7)/Mas axis could lead to new pharmacological treatments for hypertension and related cardiovascular, renal, cerebral, and metabolic syndromes.

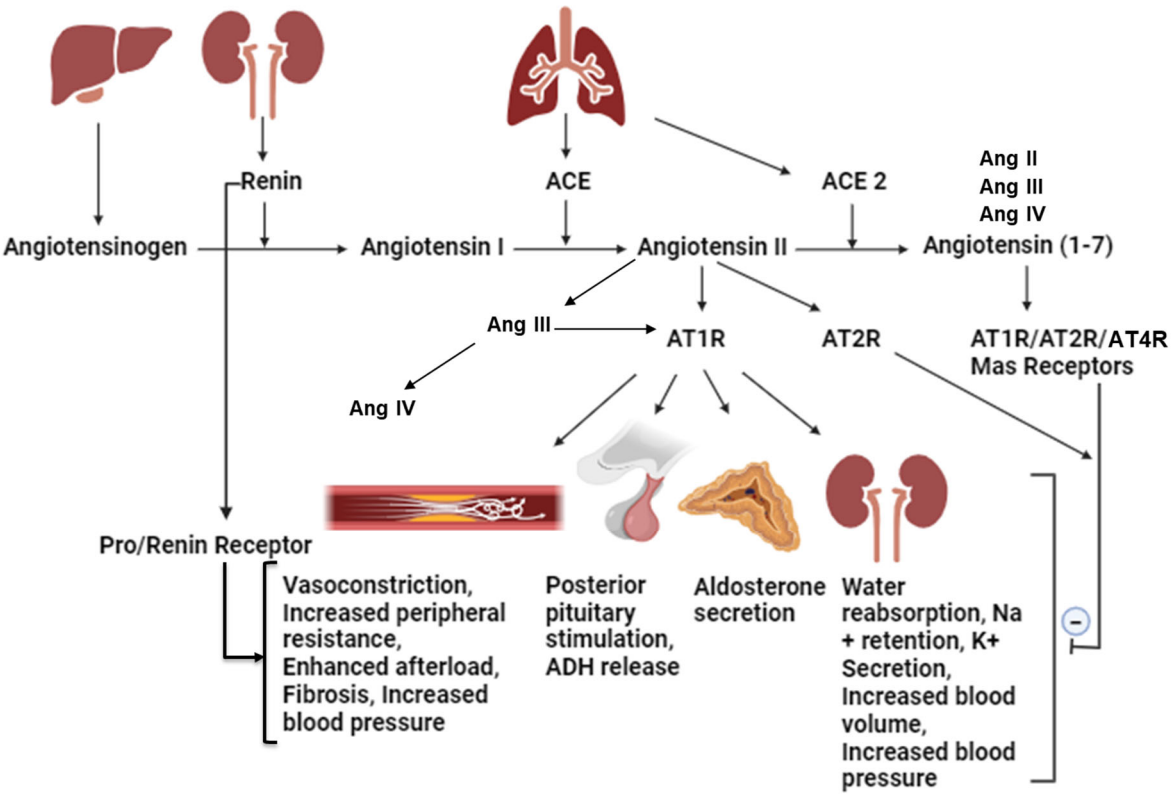
Keywords: RAS, Ang II, Ang 1-7, ACE, ACE 2, AT1R, AT2R, Mas receptor

Introduction

We are living in a rapidly changing world that has led to the globalization of unhealthy lifestyles. Non-communicable diseases such as cardiovascular disease (CVD), cancer, diabetes, and chronic lung diseases have overtaken infectious diseases being the leading cause of mortality worldwide [1]. One of the key risk factors for CVD is hypertension. Hypertension affects billions worldwide, leading to heart attacks and strokes, and currently kills nine million people every year [2]. Hypertension is a silent, invisible killer that is asymptomatic, and hence awareness aids early detection while self-care helps ensure regular intake of medication, healthy behaviors, and better control of the condition [3]. In contrast, many developing countries are seeing growing numbers of people with hypertension mainly with the population of adults aged 25 and above [4,5].

Blood pressure (BP) is created by the force of blood pushing against the walls of blood vessels (arteries) as it is pumped by the heart [6]. Hypertension is a condition in which the blood vessels have persistently raised pressure [7]. The higher the pressure in blood vessels the harder the heart has to work to pump blood [8]. If left uncontrolled, hypertension can lead to a heart attack, enlargement of the heart, and eventually heart failure [9]. Blood vessels in turn become bulged and weak,

Graphical Abstract



making them more likely to clog and burst. The pressure in the blood vessels can also cause blood to leak out into the brain leading to stroke [10]. Hypertension can also lead to kidney failure, blindness, rupture of blood vessels, and cognitive impairment [11]. One of the major pathways contributing to hypertension is the renin angiotensin system (RAS) [12].

RAS regulates homeostasis in the biological system that involves multiple biologically active molecules exhibiting varied responses [13]. Very recently the pathway has been categorized into classical or conventional and non-classical or non-conventional pathways [14,15]. In brief, the classical pathway involves the reactions of conversion of angiotensinogen (Ang) to angiotensin I (Ang I) by the action of renin. Ang I is then converted to angiotensin II (Ang II) by the action of the angiotensin converting enzyme (ACE) [16]. Ang II then acts on angiotensin II type 1 receptor (AT1R) leading to various undesirable hypertensive responses such as vasoconstriction, proliferation, hypertrophy, fibrosis, oxidative stress, central nervous system (CNS) stimulation, the release of aldosterone and anti-diuretic hormone (ADH) and, so on [17,18]. Other non-significant catabolites of Ang II include angiotensin III (Ang III) and angiotensin IV (Ang IV). Ang III acts on AT1R and Ang IV acts on the angiotensin II type 4 receptor (AT4R) or insulin-regulated aminopeptide receptor (IRAP) to cause hypertensive responses. Ang III and IV are further cleaved into the biologically inactive peptides through the action of endopeptidases [19,20]. Therefore, the ACE/Ang II/AT1R axis also termed the conventional or classical RAS pathway occurs abundantly in the physiological system resulting in hypertension and other undesirable pathophysiological conditions [21]. Hence, RAS has been a major target for therapeutics to combat hypertension and related pathophysiology, specifically with an emphasis on ACE inhibition which is also the current treatment option available in the market [22]. As already mentioned, very recently, RAS has been categorized into two distinct pathways, due to the identification of new moieties namely angiotensin converting enzyme 2 (ACE 2), angiotensin (1-7) [Ang (1-7)] and Mas receptor which is a proto-oncogene [23]. It was not until the year 2000, that the interaction of Ang (1-7) with Mas was established and Mas was proved to be a G-protein coupled receptor (GPCR) [24]. These molecules exhibit completely contradicting effects that lead to lowering hypertension with vasodilation, nitric oxide (NO) release, anti-fibrosis, anti-arrhythmia, anti-proliferation, anti-remodeling, and anti-thrombotic effects [15]. The non-conventional pathway of RAS which includes the ACE 2/Ang (1-7)/Mas axis, plays a pivotal role in maintaining normal blood

pressure in cardiovascular, renal, and, other physiological systems as a natural endogenous combat mechanism against the conventional axis [25].

The heptapeptide Ang (1-7) formed from Ang I or Ang II through the action of ACE 2, independent of ACE was first described by Santos *et al.* [26]. Schiavone *et al.* later described the effects of Ang (1-7) *in vitro* to release vasopressin from hypothalamus-hypophyseal explants [27]. Further, Chapell *et al*. (1989) showed the presence of heptapeptide in rat brains [28]. Also, Campagnole-Santos *et al.* studied the first *in vivo* action of Ang (1-7). Ang (1-7) is further cleaved into the biological inactive peptides through the action of various endopeptidases. These studies led to the recognition of Ang (1-7) to be an important moiety as the biologically active peptide in RAS [29].

Another modicum to plays a crucial role in the non-conventional RAS is ACE 2. Sharing about 60% sequence homology with ACE, ACE 2 forms angiotensin 1-9 [Ang (1-9)] from Ang I by cleaving leucine (leu) from the carboxy-terminal (c-terminal) [30]. The biologically active Ang (1-9) is subsequently cleaved to Ang (1-7) by the action of ACE and natriuretic endopeptidase (NEP) [31]. A more physiologically and biologically convenient mechanism for Ang (1-7) production is through the cleavage of phenylalanine (phe) from the c-terminal of Ang II through the action of ACE 2 [32]. It is also reported that the membrane-bound ACE 2 (mACE 2) present in the lung epithelium, serves as an entry point for certain viruses including HCoV-NL63, SARS-CoV, and SARS-CoV-2. The SARS-CoV-2 spike protein itself is known to damage the endothelium via downregulation of ACE 2 [33,34]. The other enzymes involved in the aforementioned reaction of cleaving Ang I and Ang II to Ang (1-7) are NEP, prolyl-carboxypeptidase, prolyl-endopeptidase and thimet oligopeptidase, where NEP contributes the majority of plasma Ang (1-7) concentration [31]. Identification of this monocarboxypeptidase, ACE 2 has led to the unraveling of the existence of a completely different enzymatic mechanism of RAS as the endogenous regulator of hypertension [35,36]. This is because Ang II which is a vasoconstrictor and a proliferative peptide when acts through AT1R, is degraded to Ang (1-7) which is a vasodilator and an anti-proliferative peptide, with the desired anti-hypertensive effects produced within the physiology through the Mas receptor [37].

The final iota that is essential to the non-classical RAS is the receptor. Mas stands for mitochondrial assembly 1, isolated from *Saccharomyces cerevisiae* which accumulates mitochondrial proteins and was named MAS 1. Later in 1986, in the human DNA of the epidermoid carcinoma cells, a gene named MAS 4 was identified that was a proto-oncogene. There is no correlation between MAS 1 and MAS 4 anyhow. Therefore, the nomenclature was finalized to Mas related to RAS with no other expansion terminologies [38,39]. Only in the early 2000s, the receptor was characterized to be a GPCR and the ligand was deduced to be Ang (1-7). Evidence was previously available though, where Ang (1-7) is bound to a different receptor apart from AT1R and AT2R using the Ang (1-7) antagonist. The Mas receptor exerts various beneficial effects that can counteract the deleterious effects caused by the ACE/Ang II/AT1R axis. Thereby proving that the ACE 2/Ang (1-7)/Mas receptor axis is the essential endogenous system in averting the hypertensive pathophysiology [40].

There is also a new mechanism through which anti-hypertensive effects of RAS are brought about where Ang (1-7) can be converted into alamandine. The process is through the decarboxylation of the aspartic acid (asp) moiety of Ang (1-7) to yield alanine (ala) residue. Ang II can also be catabolized into angiotensin A (Ang A) through the decarboxylation process by the action of ACE 2 which is further cleaved into alamandine. Alamandine acts on the Mas-related G-protein coupled receptor D (MrgD) to bring about vasodilation. This is the newest addition to the non-conventional RAS pathway [41]. Also, a non-RAS mechanism to metabolize angiotensinogen to angiotensin (1-12) [Ang (1-12)] through the activity of the enzyme, chymase, is newly discovered as well. Ang (1-12) can further be processed into Ang I or Ang II through chymase again to bring about the effects of the conventional RAS [42]. Studies have also represented the complex cross-talks between apelin and sirtuins with RAS to regulate CVDs [43-46].

The metabolic pathway representing RAS is represented in **(Figure 1)** which portrays the detailed proteolytic degradation of a huge polypeptide Ang I to various biologically active conventional and non-conventional axes peptides and further to the biologically inactive peptide fragments.

Varied responses are produced when different effectors act on different receptors belonging to RAS. Renin and prorenin also contain a specific pro/renin receptor, that also contribute to the hypertensive effects in the physiology. Ang II acts on the AT1R to bring about various pathophysiological responses such as vasoconstriction, increased blood volume, increased peripheral resistance, enhanced cardiac afterload, increased BP, Na^+ and water reabsorption, aldosterone and ADH secretion, CNS and posterior pituitary stimulation, and so on [3]. Agonism of AT1R basically, leads to hypertension and other cardiovascular complications. Whereas the same molecule, Ang II when acts on angiotensin II type 2 receptor (AT2R), anti-hypertensive responses such as vasodilation, anti-proliferation, pro-apoptosis, etc. Ang II further metabolized into Ang III can act on the AT1R to lead to hypertension and AT2R to produce anti-hypertensive effects. Similarly, Ang III is catabolized into Ang IV which acts on the AT4R to bring about vasodilation, neuronal development, cell proliferation, and anti-inflammation. AT4R is the insulin-regulated membrane aminopeptidase which is selective for Ang IV [20]. In brief, the physiology itself has counterbalancing effects and AT1R

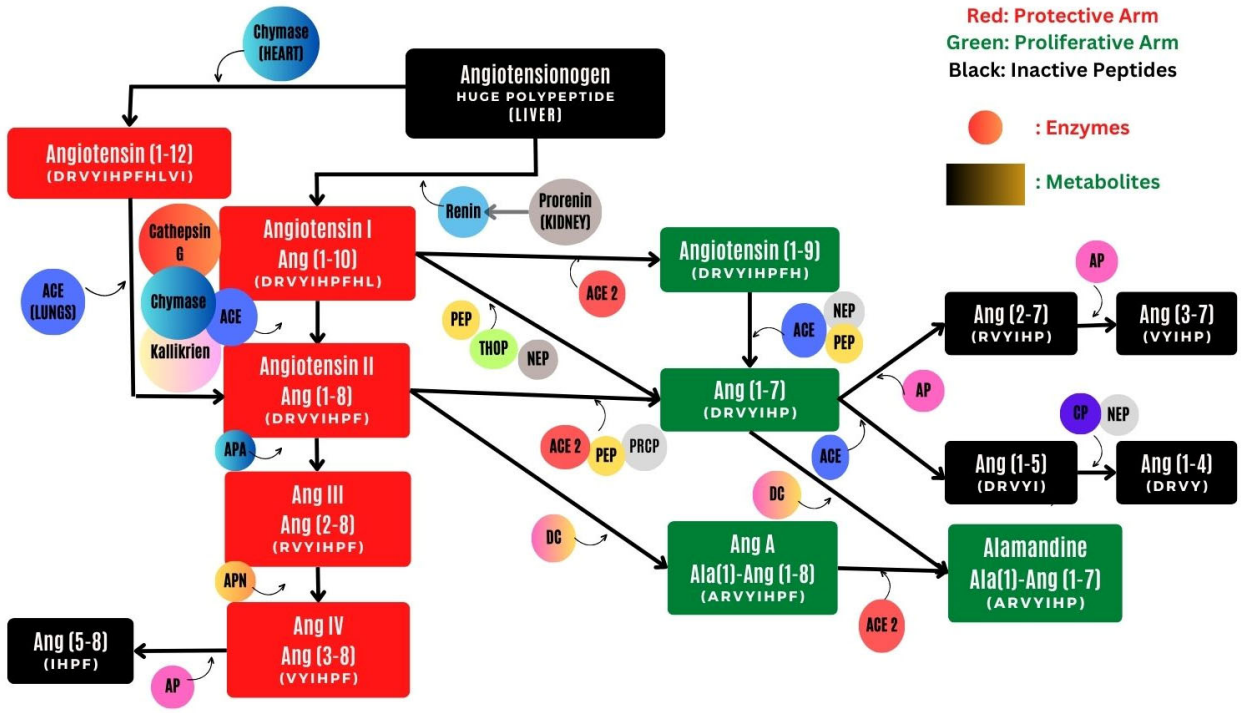


Figure 1. Representation of the RAS metabolic pathway consisting of both conventional and non-conventional arms which produce hypertensive and non-hypertensive responses respectively along with the chymase and the alamandine pathways. Moieties in red and green are the components of conventional and non-conventional arms respectively. Moieties in black represent biologically inactive molecules.

leads to hypertension, and AT2R and AT4R oppose the effects of AT1R [19]. However, AT1R is very potent and consists of more affinity towards the ligands and thus overpowers the beneficial actions of AT2R and AT4R. Ang (1-7) produced through ACE 2, either from Ang I, Ang II, or Ang (1-9) acts upon and agonizes Mas receptors and leads to anti-hypertensive responses such as vasodilation, anti-proliferation, anti-fibrosis, and fluid volume homeostasis. Therefore ACE/Ang II/AT1R are termed the hypertensive and the conventional axis of RAS, whereas ACE 2/Ang (1-7)/Mas is thereby the non-conventional axis of the RAS [47]. The varied effects of different ligands on the receptors are represented in **(Figure 2)** which will be discussed in the further sections in detail. This review focuses on the cardiovascular, renal, and metabolic role of the ACE 2/Ang (1-7)/Mas receptor axis in the physiological system, and Ang (1-7) interactions with AT1R, AT2R, and the Mas receptor.

Ang II and AT1R

As aforementioned in detail, the ill effects of Ang II through the AT1R leading to pathophysiology concerning CVD, and the subcellular molecular pathways that are activated are to be discussed as follows. AT1R expression is predominant in the vascular smooth muscles, endothelium, cardiac and renal tissues [3]. AT1R shows higher affinity towards Ang II and therefore activates the conventional axis of RAS. Tyrosine^{4} (tyr^{4}) and phenylalanine^{8} (phe^{8}) of Ang II interacts with asparagine^{111} (asn^{111}) and histidine^{256} (his^{256}) of AT1R respectively. These associations lead to the conformational changes in the transmembrane 3 and 6 helices of the receptor. Successively, transmembrane helices 3 and 6 associate with transmembrane helices 2 and 7 respectively. This activates the cytosolic signaling cascades mediating vascular smooth muscle cell (VSMC) remodeling, hypertrophy, and migration. The G-proteins associated with AT1R are $G_{q/11}$, G_{i}, G_{12}, and G_{13}. These associated proteins produce secondary messengers such as inositol 3-phosphate (IP_3), diacylglycerol (DAG), and reactive oxygen species (ROS) along with activation of phospholipase C β (PLC β) and Pho guanine nucleotide exchange factor (GEFs). These mediators bring about vasoconstriction and contraction of VSMC via myosin light chain kinase activation or inhibition of the myosin light chain phosphatase. When there is no phosphorylation of the myosin light chain, contraction takes place. Ang II when binds to AT1R, also activates nicotinamide adenine dinucleotide phosphate hydrogen (NADPH) oxidase, growth factor (GF) receptor transactivation, direct interaction with Janus kinase 2 (JAK 2), phospholipase C γ 1 (PLC γ 1), AT1R associated protein (ATRAP), ATRAP 1, and GEF. AT1R signaling

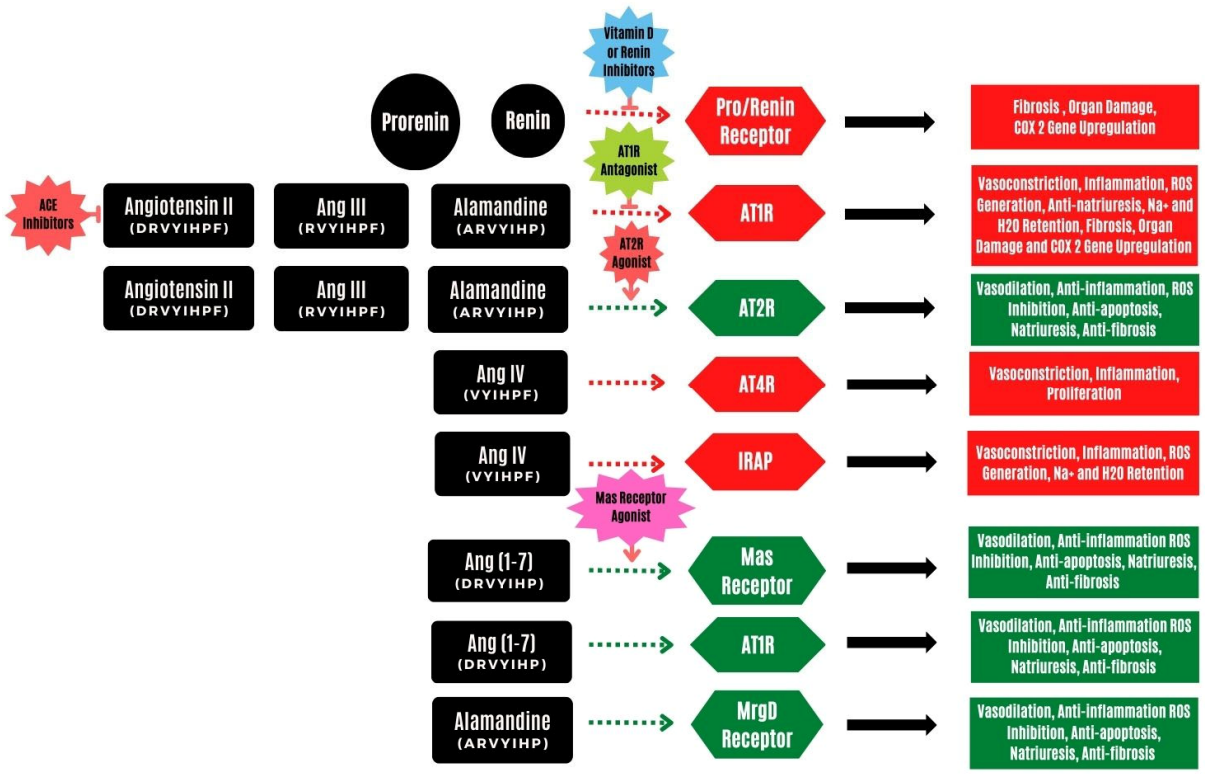


Figure 2. Representation of various RAS effector molecules acting on various RAS receptors to bring about varied responses. Moieties in red and green are the hypertensive and the anti-hypertensive responses respectively. Moieties in black represent the peptide molecules. The central hexagonal columns represent the receptors involved in RAS followed by the responses they yield. Star-shaped moieties are the representation of target areas for therapeutic interventions.

is highly complex which also includes β-arrestin signaling and interaction of many other proteins which further activates ERK 1/2 and Ak strain transforming (Akt) pathways. This deduces the fact that AT1R signaling cross-talks with other signaling cascades representing the functional significance in CVDs [48]. In brief, Ang II when acting upon AT1R, activates the voltage-gated Ca^{2+} channels, phospholipase C (PLC), phospholipase D (PLD), and phospholipase A_2 (PLA_2). Activated PLC produces IP_3 and diacylglycerol (DAG) which act on the receptors present on the endoplasmic reticulum (ER) which further opens up the Ca^{2+} channels resulting in Ca^{2+} efflux. Ca^{2+} then binds to calmodulin further activating the myosin light chain kinases. Thus, phosphorylating the myosin light chains and aiding smooth muscle contraction [2,3,18].

Ang II and AT2R

Ang II when binds to the AT2R, vasodilation, and other anti-hypertensive effects are produced due to protein kinase A (PKA)- dependent nitric oxide synthase (NOS) activation. Alternatively, or parallelly paracrine signaling is induced by bradykinin (BK), NO, and cyclic guanosine monophosphate (cGMP) production. This leads to natriuresis, reduced BP, vasodilation, and a reduction in Ang II-mediated hypertension [49].

Ang (1-7) as an AT1R Antagonist

Ang (1-7) binds to AT1R and antagonizes the pressor effects of Ang II. Ang (1-7) binds to AT1R when the concentration is elevated (≥ 10^{-6} mol/L) by competing with Ang II along with having more affinity towards the receptor (K_i = 8.0±3.2 nM). Ang (1-7) does not affect the basal Ca^{2+} levels but increases the Ang II and Ang IV dependent Ca^{2+} release in the mesangial cells at pharmacological concentrations [50]. At physiological concentrations, Ang (1-7) exhibits endothelial modifications of AT1R-mediated responses at an intracellular level. The Ang II-induced activation of protein kinase C (PKC) and ERK 1/2 in VSMC was also antagonized by the presence of Ang (1-7). In CHO cells transfected with Mas and Hirschsprung-associated enterocolitis (HAEC), Ang (1-7) brought about the inhibition of Ang II-induced ROS generation and c-Src, ERK 1/2, and Src homology region 2 (SH2)-containing protein tyrosine phosphatase 2 (SHP-2) stimulation. Even in the cultured rat aortic cells or simply rat cardiomyocytes, Ang (1-7) inhibited the Ang II-mediated stimulation of ERK 1/2. That can be abolished by transfecting the cultured cells with anti-sense oligonucleotide to Mas. In VSMCs, protein kinases are activated through cyclic adenosine monophosphate (cAMP) which is produced due to prostacyclin that is also inhibited by the presence of Ang (1-7) [51].

In proximal tubular cells, Ang II stimulates phosphorylation of mitogen-activated protein kinase (MAPK) such as p38, ERK 1/2, and c-Jun which is inhibited by Ang (1-7), which can be nullified in the presence of any Mas antagonist. Ang (1-7) and Mas-dependent inhibition of Ang II-dependent epidermal growth factor 2 (EGF 2) transactivation can be demonstrated in the rat VSMCs. Ang (1-7) can also modulate the effects brought about by Ang II at the molecular level during AT1R mRNA synthesis and translation which was proved by stably transfecting the CHO cells with AT1R and Ang (1-7) that significantly brought down AT1R expression. This is also true with rat aortic cells and cortical tubule interstitial area of the kidneys. Although, in the presence of Ang (1-7) or cells over-expressing Mas, AT1R mRNA expression drastically up-regulates. AT1R and Mas can undergo direct physiological interactions to exhibit the antagonistic activity of the AT1R. Nonetheless, the nature of the interaction between the two is yet to be deduced [52].

Ang (1-7) and AT2R

Ang (1-7) has a very low affinity towards AT2R whereas the Ang (1-7) effects are not even hampered by the presence of AT2R antagonists. AT2R belongs to the GPCR superfamily with the molecular weight of 41 kDa. It shows a 31% similarity to AT1R. Although in various cell sites and tissues, AT2R is associated with Ang (1-7) and influences their downstream processes. The levels of Ang (1-7) increase in the isolated mouse heart during the perfusion pressure in the presence of an AT2R antagonist where neither AT1R nor Mas was involved. One of the important observations is that the blocked AT1R unmasks the Mas-dependent vasodilatory effects of Ang (1-7) in sub-picomolar concentrations. Even in the AT2R-blocked conditions, Ang (1-7) reduces the blood pressure in the candesartan-treated spontaneously hypertensive rats (SHR) models. Certain Ang (1-7) antagonists such as A-779, PD 123319, and so on, also fail to inhibit the AT2R-dependent Ang (1-7) effects. B_2 receptor blockage surprisingly attenuates the AT2R-mediated effects. Thus, the aforementioned studies show that there is an intricate relationship between Ang (1-7), AT2R, and kinin receptors that includes various intracellular intersections via common signaling pathways that could include heterodimerization [24]. The mechanisms still are elusive. However, it is considered that activation of AT2R through Ang (1-7) occurs during pathophysiological conditions that produce short-term inhibitory effects during cardiac or renal injuries. Physiological interactions of Mas, AT2R, and B_2 receptors are only seen in certain specific tissues that can be explained through the use of specific receptor antagonists [53].

Ang (1-7) and Mas Receptor

Ang (1-7) acting upon the Mas receptor primarily produces the effects of the non-conventional RAS. Mas being a GPCR, consists of the $G\alpha_s$ subunit. $G\alpha_s$ stimulation further aids NO production through the phosphorylation of serine^{1177} (ser^{1177}) of endothelial nitric oxide synthase (eNOS). It also leads to moderated ERK signaling and stimulates cancer patients' cAMP release and hematopoietic recovery. These responses in turn attenuate cardiac hypertrophy and fibrosis that is caused by the conventional RAS [54]. However, the role of the coupled G-protein to the Mas receptor is controversial. Studies suggest that Mas is constitutively active independent of the G-protein even without the ligand binding. Although, emphasis in this regard needs attention, and further studies [55].

Cardiac Implications of ACE 2/Ang (1-7)/Mas Axis

Ang (1-7) is generally produced in the heart from Ang II as a major substrate through the action of ACE 2 which is an important modulator involved in cardiac functionality and pathophysiology that is different from species to species and is also strain-specific. In humans, the ACE 2 expression is enhanced in a failing heart ventricle that is not seen in other species. Within the cardiac tissues, the expression profile of ACE 2 is distinctive. For instance, during myocardial infarction (MI), ACE 2 expression increases in the plasma and left ventricles whereas post-MI or in normal conditions, there is a reduction in the ACE 2 expression and the mRNA levels [56,57].

The heart is an important target for the action of Ang (1-7) along with the other non-conventional axis components. Ang (1-7) shows the functional and biochemical alteration that involves vasodilation in the arterial rings, BK potentiation, or contradictory actions against Ang II. The presence of NO can have a positive influence on the effects exerted by Ang (1-7). Ang (1-7) also shows dose-dependent vasodilatory responses. Therefore Ang (1-7) actions are dependent on Mas, interaction with AT1R and AT2R, NO, and prostaglandin (PG) release in the cardiac tissues [58]. Ang (1-7) levels drastically reduce based on the incidence and the duration of ischemia and reperfusion arrhythmia [59]. Along with providing mechanical and morphological support in the sinoatrial cells, Ang (1-7) also activates Na^+ pumps and inhibits Na^+-K^+-Mg^{2+} adenosine tri-phosphatase (ATPase) activity. Ang (1-7) helps the release of PGs, BK, and Mas expression and improves post-ischemic contractility of the heart. However, an overdose of Ang (1-7) at more than a 10-fold range leads to cardiac reperfusion arrhythmia and sudden death. Chronic infusion can improve coronary perfusion and help preserve cardiac functions. Very high local concentrations of Ang (1-7) can lead to lethal deleterious complications due to NADPH oxidase activity that in turn leads to norepinephrine release. However, during MI, chronic infusion of Ang (1-7) leads to enhanced cardiac output and stroke volume. There is also a slight but significant increase in daily and nocturnal dρ/dT (ratio of pressure change in ventricular cava during the isovolumic contraction period) [60]. There is also a reversal in the vascular endothelial dysfunction in aortic rings. The immunoreactivity of Ang

(1-7) however increases in the infarct areas. Attenuation of hypertrophy and fibrosis in the heart and prevention of end-organ damage in the heart occurs due to the long-term Ang (1-7) treatment. Studies also represent improved heart functions, cardiac remodeling, and reduction in MI incidences in case of oral administration of non-digestible oligosaccharide matrices such as hydroxyl propane β-cyclodextrin (HPβCD) enclosed Ang (1-7) along with Mas agonism and ACE 2 activation [61]. Ang (1-7) greatly regulates extra-cellular matrix (ECM) protein expression and cardiac remodeling by binding to the cardiac fibroblast cells. Reduction in the levels of Ang II leads to lower levels of collagen content through inhibition of serum extra-cellular signal-regulated protein kinase 1/2 (ERK 1/2), MAPK stimulation, and, Mas activation. Chronic administration of Ang (1-7) results in alteration of left ventricular hypertrophy and fibrosis in pressure overload or Ang II-infused hypertensive models [59].

Mas expression is also based on the nature and duration of the pathophysiological stimuli. Mas deficiency leads to impaired heart function, endothelial dysfunction, increased BP, reduced spermatogenesis and baroreflexes, metabolic syndrome-like state, altered thrombogenesis, and, increased cardiac ECM. ACE 2 activation can significantly reduce ERK 1/2 expression. Mas deficiency shows enhanced collagen I and III and, fibronectin levels [48].

Although the Mas mechanism in cardiac tissues is not completely characterized, Ang (1-7) shows NO production by eNOS activation and, Akt antibodies in cardiomyocytes that further potentiate BK and reduce Ang II levels.

Thus, these mechanisms convey that the non-conventional arm of RAS is a major contributor to cardio-protection in the physiological system endogenously [52].

Vascular Implications of ACE 2/Ang (1-7)/Mas Axis

One of the important sites of Ang (1-7) action is blood vessels. Ang (1-7) production and metabolism take place in the vasculature [62]. The non-conventional axis of RAS is the major producer of vasodilators such as PGs, NO, and endothelium-derived hyperpolarizing factors that altogether elicit relaxation effects in the vascular beds. ACE 2 activation alone, irrespective of ACE inhibition or AT1R blockage, reduces BP in normotensive and hypertensive mice. Although ACE inhibition might play an important role in maintaining pressure homeostasis in the vascular system. There are various other mechanisms involved in Ang (1-7) activation in the blood vessels where various factors such as the diameter of the vessels, vascular bed, and species are dependent. It is shown that there is a complex interaction of Mas, BK B2, AT1R, and AT2R with Ang (1-7). In some vessels, BK can be potentiated due to ACE inhibition or Ang (1-7) production in both normotensive and hypertensive conditions because Ang (1-7) is considered to be a substrate for ACE as well, that causes allosteric changes in ACE and Mas mediated changes in BK signaling. The vascular effects of Ang II are also brought down in the presence of Ang (1-7) [63].

Mas is predominantly found in the vascular system and is a major contributor to the regulation of pressure in the blood vessels and endothelial cells. Although Ang (1-7) can exert vasodilation on endothelium and vasculature in the absence of Mas. Mas positively influences NO release as well through Ang (1-7) mediated coordinated phosphorylation and dephosphorylation of serine^{1777} (ser^{1777}) and threonine^{495} (thr^{495}) of eNOS which is the stimulation site and inhibitory site of eNOS respectively, which finally leads to the activation of phosphoinositide 3-kinase (PI3K) -Akt pathway [64].

Renal Implications of ACE 2/Ang (1-7)/Mas Axis

The non-conventional axis of RAS imparts the renal implications of the physiological system causing hypertension. Ang (1-7) can be detected in urine. It can be seen in reduced concentration in patients with untreated essential hypertension than in normal individuals [50]. The enzyme for the production of Ang (1-7) is abundantly found in the kidneys. Ang (1-7) and angiotensin (1-4) [Ang (1-4)] are found in the renal cortex due to the abundance of ACE 2 and NEP from the substrates Ang (1-9) or Ang I. NEP is essential in the degradation of Ang (1-7) to Ang (1-4) that is a biologically inactive peptide [31]. The action of ACE 2 can be seen in circulation as well and is also predominantly produced in the proximal nephron. It is found to be 20% more effective in renal tissues than cardiac tissues for reasons still undiscovered. ACE 2 is active in the cortex region of the kidneys, and the mRNAs are expressed in the medulla region and not in the medullary thick ascending limb of the Henle's loop and outer and inner medulla. Therefore, it is highly questionable how ACE 2 is abundant in the cortex region [65]. It is shown that scavenger receptor megalin can modulate the expression of ACE and ACE 2 in the proximal tubules by Pohl *et al*. The absence of megalin leads to enhanced expression of ACE 2 and reduced expression of ACE. Thereby representing that the megalin or megalin-related pathways regulate the expression of both enzyme isoforms in the proximal tubules [47].

Ang (1-7) induces MAPK via AT1R. Ang II also induces ERK 1/2 activation via NADPH oxidase activation or epithelial growth factor receptor (EGFR) transactivation while Ang (1-7) phosphorylates ERK 1/2 in a cAMP/PKA- dependent manner to inhibit the aforementioned pathway as Mas is coupled with $G_{sα}$ [59].

It is noteworthy that the effects of Ang (1-7) in the renal tissues are highly complex and controversial. Unlike Ang II, Ang (1-7) shows increased diuretic and natriuretic effects by the inhibition of Na^+ reabsorption in the proximal tubules thereby limiting the transcellular Na^+ flux by modulating transporter activity via phospholipase A_2 (PLA_2) activation in

the tubular epithelial cells. Studies also represent that in the presence of AT2R agonist in a dose-dependent manner, the Na^+-K^+ ATPase is not inhibited in the renal cortex and isolated convoluted proximal tubule that proves that Ang (1-7) acts via AT2R in this region and not the other specific receptors [20,66]. Whereas, Ang (1-7) opposes the actions of Ang II on the Na^+-K^+ ATPase in the proximal tubule via the Mas receptor. Ang (1-7) is also an anti-natriuretic and anti-diuretic molecule leading to a reduction in the urinal volume, in the water-loaded rat models that involve Mas activation, although all three receptors, AT1R, AT2R, and Mas could be involved. In pregnant rat models, Ang (1-7) is shown to have a diuretic effect through the downregulation of Aquaporin-1 which is completely contradictory in the virgin rats where no such effects are seen. Ang (1-7) brings down glomerulosclerosis in the glomerular nephritic conditions. These contradictory behaviors suggest that the action of Ang (1-7) depends on the concentration, nephron segment, species, level of RAS activation, and water and salt status in the physiology. Thereby, RAS depicts an intricate regulatory mechanism that is way more complicated than expected [47].

Mas expression in the kidney is predominant. Mas mRNA is found in the cortex area but is expressive and functional in the proximal tubules, afferent arteriole, collecting duct, and thick ascending limb of Henle's loop. Under ischemic conditions, the expression of Mas upregulates. Mas deletion leads to enhanced renal hypertension and oxidative stress, which is reversed in the presence of an anti-oxidant. It can also lead to renal dysfunction, enhanced glomerular tuft diameter, and increased levels of fibronectin and collagen III and IV deposition along with AT1R and tumor growth factor-β (TGF-β) expression. The use of Mas agonist can also bring down renal injuries [67].

ACE 2 also shows significant anti-hypertensive responses in renal tissues. Chronic inhibition of ACE 2 in either healthy or diabetic mice leads to glomerular injury, albuminuria, and enhanced activity of ACE. Subtotal nephrectomy shows downregulation of ACE 2 in the cortex area [68]. Supplementation of recombinant ACE 2 reduces fibrosis, glomerular mesangial matrix, smooth muscle actin, and collagen III expression. ACE 2 knock-out (KO) and Ang II Wistar rat models, Collagen I-dependent gene expression related to fibrosis, like smooth muscle actin, TGF-β, and procollagen I through ERK 1/2 activation and protein kinase C (PKC) levels were enhanced. Thus, proving that ACE-ACE 2 balance, if disrupted results in renal pathophysiology [49].

Surprisingly in certain physiological conditions, the non-conventional RAS can also pose deleterious effects. For example, in Mas KO reno-insufficient models, there was attenuation of renal damage. In the absence of Mas, the renal injury was not aggravated during kidney ischemia-reperfusion [69]. Ang (1-7) led to the nuclear factor kappa B (NF-κB) activation and inflammation via Mas along with subtotal nephrectomy independent deleterious cardio-vascular effects and enhanced fibrosis. Ang (1-7) can either inhibit or stimulate epithelial transformation to mesenchymal tissues in the tubular cells. There was increased albuminuria yet reduced expression of marker genes for diabetic retinopathy in ACE 2 KO models. These conflicting reports show that the role of non-conventional RAS in renal tissues is dependent on cell type and Ang (1-7) signaling in the kidneys. This highlights the point that further in-detail studies related to the non-conventional arm of RAS and its reno-protective and reno-derogatory responses need to be characterized and could be a novel axis [70,71].

Metabolic Actions of ACE 2/Ang (1-7)/Mas Axis

RAS can be found in the endocrine and exocrine pancreas and also adipose tissues that are regulated by the food intake pattern. Enhanced sugar levels increase angiotensinogen, ACE, and AT1R levels in the pancreas. Enhanced sugar and fat levels in adipose tissues, increase ACE 2 and Ang (1-7) levels. RAS and insulin resistance are interrelated due to insulin resistance imparting cardio-vascular impairments. RAS inhibition through either ACE inhibition or the use of an AT1R antagonist can reduce hyperglycemia. Similarly, elevated ACE 2 reduces hyperglycemia in diabetic rats [55]. Ang (1-7) along with BK significantly brings down hyperglycemia while Ang II can do the opposite of the same. Ang (1-7) plays a major role in the attenuation of metabolic syndromes, enhancing glucose uptake and also protecting the cells against oxidative stress that can induce insulin resistance. There is positive regulation of insulin in the cardiac tissues, especially human endothelial aortic cells (HAEC), through the molecular mechanism that includes phosphorylation of insulin downstream effectors and PI3K and AKT activation via Mas in HAEC and insulin receptor substrate-1 (IRS-1) and Janus kinase 2 (JAK 2) via AT1R interaction. Ang (1-7) and Mas counteract Ang II and AT1R signaling in HAEC by dephosphorylation of cellular sarcoma kinase (c-Src) and ERK 1/2 and inhibition of NADPH oxidase activity [72]. The phosphoproteome study of Ang (1-7) in human endothelial cells represents a novel Ang (1-7)/Mas axis that indicates the interdependence of Ang (1-7) with insulin and lipid metabolism. In a recent study on TGR [Ang (1-7)] 3292 animal models, normal food intake showed increased plasma Ang (1-7) concentration that further reduced fat mass, triglycerides (TGs), and cholesterol. There was an increase in the expression of adiponectin and adipose lipid binding protein (AP 2) and a decrease in angiotensinogen expression. Adiponectin, a key adipokine and an essential protein in adipose tissue metabolism and fatty acid esterification, regulates insulin sensitivity and tissue inflammation is found in lower amounts in plasma and enhances the body fat content [73]. Ang (1-7) and Mas axis have also been shown to involve a complex cross-talk with Sirtuins and Apelins to lead to reduced adipose tissue mass in high-fat-fed diet animal models. This

showed to reduce the levels of ACE and enhanced ACE 2 levels further leading to reduced metabolic syndromes such as central obesity, insulin resistance, hyperlipidemia, hyperglycemia, and hypertension. Therefore, Ang (1-7) and Mas, given their role in carbohydrate and lipid metabolism through the inhibition of the conventional axis can improve the metabolic profiles in the physiological system [43]. Sirtuins are a class of peptide moieties present and conserved from bacteria to mammals and act as the class III nicotinamide adenine dinucletide (NAD^+)-dependent histones (functional deacetylase or mono-ADP ribosyl transferases). Sirtuins are essential moieties in gene silencing, metabolism, stress regulation, and anti-aging effects in response to fasting. This represents that anorexic stimuli are brought about by the non-conventional axis of RAS that can help combat metabolic syndromes including obesity [44]. Apelins are a group of oligopeptides as well that regulate obesity, diabetes, neuroendocrine functions, energy metabolism, and kidney disorders. The apelin levels are determined by lifestyle, diet, and exercise routines. This proves that induction of non-conventional RAS and suppression of the conventional RAS depends on the individual [46]. However, as mentioned, RAS is highly complex and differs from species to strain. Chronic diseases and neurodegeneration are growing concerns worldwide. These conditions are influenced by central neuroendocrine and RAS dysregulation, which can be affected by stress, diet, and lifestyle. Such factors disrupt NO and neural pathways, leading to appetite dysregulation and organ disease. Environmental pollutants and unhealthy diets exacerbate these issues by increasing xenobiotics and oxidative stress, which contribute to neuroendocrine disorders, kidney disease, and non-alcoholic fatty liver disease (NAFLD). Increased oxidative stress and poor apelin and RAS regulation, combined with impaired nitric oxide and vascular Aβ metabolism, accelerate the development of obesity, diabetes, cardiovascular disease, and neurodegenerative conditions [44-46].

ACE KO reduces fat mass due to increased lipid metabolism and energy expenditure along with upregulating the key gene expression of hydrolysis of lipids in free fatty acids such as lipoprotein lipase, fatty acid translocation to mitochondria such as carnitine, palmitoyl transferase 1 (PT 1) and β-oxidation inside mitochondria and peroxisomes such as long chain acyl dehydrogenase. Mas KO leads to impaired lipid metabolism that can further lead to dyslipidemia, reduced glucose tolerance, decrease in adiponectin secretion, hyperinsulinemia, reduced insulin sensitivity, enhanced abdominal fat mass compared to the wild-type, etc [54].

ACE 2 helps protect against high calorie diet induced insulin resistance through GLUT-4 via Ang (1-7) dependent pathways as well [43].

Therefore, RAS is the potential target for metabolic syndrome treatment that includes obesity, insulin resistance, hypertension, dyslipidemia, and so on as this particular axis, ACE 2/Ang (1-7)/Mas, is essential in carbohydrate and lipid metabolism.

Conclusion

RAS complexity is beyond all the explanations deduced to date that we could even suspect a few years ago. Nonetheless, AT1R signaling transductions are a central cascade in RAS. Emerging evidence has shown that AT1R signaling cross-talks with other mechanisms resulting in cardio-vascular-associated complications. Certain new elements such as ACE 2, Mas, and Ang (1-7), prorenin receptors are added that have changed the very perception of the role of RAS in the physiological system. Ang (1-7) peptide, ACE 2, and the Mas receptor are integral components of the RAS, which plays a critical role in regulating blood pressure, fluid balance, and cardiovascular function. Research into these components has expanded our understanding of their potential therapeutic applications and implications for future treatments. Ang (1-7) has been shown to have vasodilatory effects and protective roles in cardiovascular diseases. It counteracts some of the harmful effects of Ang II, which is often elevated in conditions like hypertension and heart failure. Emerging research suggests that Ang (1-7) might play a role in metabolic processes and insulin sensitivity, potentially influencing diabetes management and metabolic syndrome. The components of the non-conventional RAS or its analogs could be developed as therapeutic agents for hypertension, heart failure, and other cardiovascular disorders. They might offer an alternative or adjunct to traditional ACE inhibitors or ARBs. There is evidence that the ACE 2/Ang (1-7)/Mas axis has neuroprotective effects, which could be leveraged in neurodegenerative diseases or stroke recovery. The interplay between Ang (1-7), ACE 2, and the Mas receptor presents a promising area for research and therapeutic development. As we deepen our understanding of these components, novel treatments for cardiovascular diseases, metabolic disorders, and even some infectious diseases may emerge. The therapeutic strategies could range from direct modulation of these pathways to innovative approaches such as gene therapy or receptor-targeted drugs. Continued research will be essential to fully realize and optimize these potential benefits. It is thereby conclusive that RAS has dual nature and the two arms of pathways are both beneficial and harmful. The ACE/Ang II/AT1R is the deleterious arm and the ACE 2/Ang (1-7)/Mas axis is the advantageous arm as per the studies summarized above in this article. The new non-conventional RAS axis is involved in the regulation of BP, metabolism, and pathogenesis of at least cardiovascular, renal, and metabolic disorders but is elusive in certain cases. Therefore, investigation of the new cardiovascular drugs harnessing the activation of the non-conventional axis of RAS is trending in the pharmaceutical industry and further in-detail studies need to be carried out to completely understand these intricate and highly complex pathways of RAS. Complete knowledge of the RAS pathways are essential to contribute to the therapeutics in hypertension and CVDs.

Abbreviations

ACE 2: Angiotensin Converting Enzyme 2; ACE: Angiotensin Converting Enzyme; ADH: Anti-Diuretic Hormone; Akt: Ak strain transforming; ala: Alanine; Ang (1-12): Angiotensin (1-12); Ang (1-4): Angiotensin (1-4); Ang (1-7): Angiotensin (1-7); Ang (1-9): Angiotensin (1-9); Ang A- Angiotensin A; Ang-Angiotensinogen; Ang I- Angiotensin I; Ang II- Angiotensin II; Ang III- Angiotensin III; Ang IV: Angiotensin IV; AP 2: Adipose Lipid Binding Protein 2; asn^{111}- Asparagine^{111} ; asp: Aspartic Acid; AT1R: Angiotensin II Type 1 Receptor; AT2R: Angiotensin II Type 2 Receptor; AT4R: Angiotensin II Type 4 Receptor; ATPase: Adenosine Tri-Phosphatase; ATRAP: Angiotensin II Type 2 Receptor-Associated Protein; BK: Bradykinin; BP: Blood Pressure; cAMP: Cyclic Adenosine Mono-Phosphate; cGMP: Cyclic Guanidine Monophosphate; CNS: Central Nervous System; c-Src: Cellular Sarcoma; c-terminal: Carboxy Terminal; CVD: Cardiovascular Disorders; DAG: Diacylglycerol; ECM: Extra Cellular Matrix; EGF 2: Epidermal Growth Factor; EGFR: Epithelial Growth Factor Receptor; eNOS: Endothelial Nitric Oxide Synthase; ER: Endoplasmic Reticulum; ERK 1/2: Extra-Cellular Signal-Regulated Protein Kinase 1/2; GEF: Guanine Nucleotide Exchange Factor; GF: Growth Factor; GPCR: G-Protein Coupled Receptor; HAEC: Human Endothelial Aortic Cells; his^{256}: Histidine^{256}; HPβCD: Hydroxyl Propane β-Cyclodextrin; IP_3: Inositol 3-Phosphate; IRAP: Insulin-Regulated Aminopeptide Receptor; IRS-1: Insulin Receptor Substrate- 1; JAK 2: Janus Kinase 2; KO: Knock-Out; leu: Leucine; mACE2: Membrane-Bound ACE 2; MAPK: Mitogen-Activated Protein Kinase; MAS 1 & 4: Mitochondrial Assembly Protein 1 & 4; MI: Myocardial Infarction; mRNA: Messenger ribonucleic acid; MrgD: Mas:related G:protein coupled receptor D; NAD^+: Nicotinamide adenine dinucletide; NADPH: Nicotinamide Adenine Dinucleotide Phosphate; NAFLD: Non Alcoholic Fatty Liver Disease; NEP: Natriuretic Endopeptidases; NF:κB: Nuclear Factor: Kappa B; NO: Nitric Oxide; NOS: Nitric Oxide Synthase; PG: Prostaglandins; phe: Phenylalanine; Phe^8: Phenylalanine^8; PI3K: Phospho-inositide 3 kinase; PKA: Protein Kinase A; PKC: Protein Kinase C; PLA 2: Phospholipase A 2; PLC: Phospholipase C; PLC β: Phospholipase C β; PLC γ 1: Phospholipase C γ 1; PLD: Phospholipase D; PT 1: Palmitoyl Transferase 1; RAS: Renin Angiotensin System; ROS: Reactive Oxygen Species; ser^{1177}: Serine1177; ser1777: Serine^{1777}; SH2: Src Homology Region 2; SHP:2: Src Homology Region 2 Containing Protein Tyrosine Phosphatase 2; SHR: Spontaneously Hypertensive Rats; TGF-β: Transforming Growth Factor β; TGs: Triglycerides; thr^{495}: Threonine^{495}; tyr^4: Tyrosine^4; VSMCs: Vascular Smooth Muscle Cells.

Conflict of Interest

The authors declare no conflict of interest.

Author Contribution Statement

Supriya Sarkar: Conceptualization, Methodology, Software, Resources, Writing- Original Draft, Visualization.

Bannikuppe Sannanaik Vishwanath: Conceptualization, Validation, Supervision, Visualization, Project Administration, Writing- Reviewing and Editing.

K. Jayachandra: Validation, Writing- Reviewing and Editing.

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