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In this article, we focus on one of the most intensely studied hormone-receptor systems in humans responsible for the regulation of blood pressure and water-electrolyte homeostasis. Since the last review chapter on Angiotensin Receptor (Inagami, 2004), there have been significant advances made in understanding the signal transduction mechanisms, pharmacology, and structural biology of these receptors as well description of a counter-regulatory arm of the renin-angiotensin system (RAS). There are four angiotensin (Ang) peptides, Ang II, Ang III, Ang IV, and Ang (1 – 7), which meet the definition of an endogenous hormone that act through specific receptors. These cell surface Ang receptors, also called “interpreters of angiotensinergic signals”, have been described in great detail in previous reviews (Forrester et al., 2018; Karnik et al., 2015). Angiotensin II, the classical blood pressure hormone binds and activates the type 1 (AT1R) and type 2 (AT2R) receptors. The Ang (1 – 7) peptide is paired with multiple receptors, of which Mas receptor (MasR) and the Mas-related receptor (MRGD) are better studied. These four Ang receptors are seven-transmembrane helical G protein-coupled receptors (GPCRs) that rely on heterotrimeric G proteins for mediating their cellular effects. These GPCRs are also capable of mediating β-arrester biased signaling. The receptors for Ang IV (AT4Rs) are not GPCRs, instead they are type II single transmembrane Zn metalloproteases, IRAP (insulin regulated aminopeptidase), and its paralog AP-N (aminopeptidase-N). Ang III, Ang (1 – 7) and Ang IV also function as surrogate ligands for AT1R and AT2R.

Careful studies have been performed by researchers for more than a century to understand several pathological states that result from RAS dysregulation in order to develop effective therapies. Indeed these efforts have resulted in two classes of highly successful and safe drugs, the angiotensin-converting enzyme inhibitors (ACEIs) and the AT1 receptor blockers (ARBs). Along with the importance of classical RAS, this article will highlight recent advancement in the field such as the role of counter-regulatory RAS, recent breakthroughs in defining the active state crystal structures of AT1R and AT2R; RAS components as targets of new diseases, the clinical potential of the approved drugs, and RAS targeting drugs under clinical trials.

**Generation of Hormone Peptides of RAS and Consequences of Inhibition**

All four angiotensin hormones are produced from the ten residues long amino-terminal segment (called Ang I) of angiotensinogen, a ≈ 65 kd serpin produced predominantly in the liver. Angiotensinogen gene knockout is lethal in mice, and it is the only source for the production of Ang I in the human proteome. The cascade of enzymes that are not localized to any one tissue but act on circulating and locally produced Ang I or its derivative fragments to generate the hormone peptides constitute RAS.

The initiating enzyme of the RAS cascade is renin, an aspartyl protease predominantly produced in the kidney that reacts exclusively with angiotensinogen, and cleaves only one peptide bond between residues 10 and 11 generating the decapeptide Ang I (Fig. 1). No hormonal action is detected for Ang I, although its ubiquitous distribution may be attributed to Ang I-binding carrier proteins that show no receptor-like function to transmit any signal. The physiological significance of Ang I is that it is the only source for the hormone-generation role of RAS in body fluids and the intra- or inter-cellular space. Many isoforms of renin exist that show tissue-specific variation in expression. Renin gene knockout in mice is not lethal.

An orally active non-peptide, a direct renin inhibitor, Aliskiren (Fig. 2) was FDA approved for the treatment of hypertension. It reduced plasma renin activity and hypertension, which are effects similar to those produced by ACEIs and ARBs. Aliskiren is well-tolerated with a relatively lower incidence of cough and angioedema (life-threatening airway swelling and obstruction) than those taking ACEIs. Gastrointestinal adverse effects upon taking Aliskiren is reported in some patients. The ALTITUDE trial studies (Novartis, 2014) reported increased adverse events (non-fatal stroke, renal complications, hyperkalemia, hypotension) with no apparent additional benefits in diabetic patients.

Release of renin by the kidney is under feedback control of circulating Ang II levels as well as many physiological factors, including ambient blood pressure, fluid volume, and electrolyte balance. Higher levels of prorenin than renin is released to plasma in disease states such as hypertension and diabetes. The question, how prorenin is activated outside the kidney led to the discovery of the prorenin receptor (PRR) (Batenburg et al., 2004; Nguyen et al., 2002). PRR is a ubiquitously expressed 350-amino acid protein, previously described as Na/H + ATPase, that binds prorenin and induces Ang I generation without proteolytic activation of prorenin. PRR expression levels are high in brain regions, which could be particularly relevant because the expression of classic RAS components is low. The PRR may contribute to angiotensin surges, and drugs to control renin activity by targeting PRR may be developed in the future.

The prohormone Ang I serves as the substrate for two prominent Zn protease angiotensin-converting enzymes, ACE and ACE2 abundantly expressed in the lungs but also in other tissues. Classical ACE is the same protein known as the kininase II in the kinin-kallikrein system. Thus, ACE converts Ang I to the vasoconstrictor octa-peptide hormone Ang II (Fig. 1), and it also degrades...
vasodilator bradykinins. ACE activity increases blood pressure by producing Ang II, which induces constriction of blood vessels. This enzyme is the target of one of the most successful antihypertensive drugs, with a minimum of side effects and complications. Starting with captopril, a series of long-acting ACE inhibitors such as ramipril, enalapril, lisinopril (Fig. 2) have been highly successful drugs, primarily used for the treatment of high blood pressure, but have been effectively repurposed for treatment in heart failure, kidney damage in diabetes and fibrotic tissue damage.

ACE2 is a late addition to RAS, after the characterization of AT1 and AT2 receptors. It is a smaller molecule with high homology and functional similarity to ACE. However, it’s enzymatic mechanism is different, being a carboxyl mono-peptidase it degrades Ang II to produce Ang (1–7) (Fig. 1). Removal of the C-terminal residues from Ang I to form Ang (1–9) combined then with the carboxyl di-peptidase activity of ACE also produces Ang (1–7) and Ang (1–5) (Fig. 1). Of these, Ang (1–9) and Ang (1–5) are inert degradation products. There are a few reports of the biological effects of these peptides that need further confirmation. Whereas Ang (1–7) was first shown to activate phospholipase A2 to release arachidonic acid from phospholipids leading to the formation of prostaglandins through a GPCR, MasR, and later through the Mas related GPCR, MRGD. Both inhibitory and potentiating pharmacological agents targeting ACE2 have been disappointing in experimental systems without leading to clinically useful drugs as yet.

In recent literature actions of Ang II through AT1R is described as classical RAS axis (Fig. 1) presumably because most of the known Ang II functions are mediated by the AT1R. Further, AT1R gene null mice do not survive, an outcome similar to knocking out the angiotensinogen gene. In contrast, knocking out genes for all other components of RAS is not lethal but demonstrate adaptive changes. Thus, the second axis of RAS, a counter regulator of the classical RAS axis (Fig. 1), is proposed consisting of AT2R, MasR, and MRGD, their cognate ligands, and enzymes that produce these ligands. We will summarize recent advances concerning these two arms of RAS.
Classical RAS, Signaling Mechanisms and Topics of Current Interest in AT₁R Research

Classical RAS consisting of ACE/Ang II/AT₁R promotes almost all of the homeostatic regulatory functions of Ang II on cardiovascular, renal, and cerebral systems. The physiological levels of the hormone Ang II maintains normal blood pressure through regulation of peripheral resistance of vasculature, heart rate, cardiac output, neuronal control, and body fluid homeostasis through activation of the AT₁R. Whereas, overstimulation of AT₁R is associated with disease states such as hypertension, heart failure, cardiac hypertrophy, coronary artery disease, stroke, ischemic heart, diabetic nephropathy, arrhythmia and renal diseases (Khan, 2011; Lee et al., 2012; Vejakama et al., 2012; Vijayaraghavan and Deedwania, 2011).

Upon Ang II binding to AT₁R intracellular signals are mediated by heterotrimeric G-proteins (Gq/11, G12/13, and Gi), which interact with the receptor, followed by the production of second messengers such as inositol trisphosphate, diacylglycerol, reactive oxygen species (ROS), and arachidonic acid. These molecules trigger the activation of downstream effectors like phospholipases C, A, and D. Furthermore, AT₁R activates various protein kinases intracellularly, including serine/threonine kinases such as mitogen-activated protein kinase (MAPK) family kinases, Protein kinase C (PKC), and Protein kinase B (PKB or Akt), receptor tyrosine kinases (RTKs) and non-receptor tyrosine kinases (nRTKs). AT₁R also produces G protein-independent signals, such as β-arrestin signaling (Wei et al., 2003). Some novel and expanding Ang II signaling pathways investigated are Wnt/ β-catenin pathway, Notch pathway, NLRP3 (Nucleotide-binding domain and leucine-rich repeat-containing PYD-3) inflammasome and Hippo pathway which plays a variety of roles during developmental processes. Further, the key expanding areas include intracellular as well as extracellular organelle signal communications, signaling through posttranslational protein modification, cellular and tissue metabolic modulation, microRNAs, and long noncoding RNAs (Forrester et al., 2018).

Non-canonical AT₁R functions have been described that include ligand-independent activation of AT₁R through either mechanical stress or AT₁R -directed agonist autoantibodies or via receptor mutations (Liu et al., 2016; Mederos y Schnitzler et al., 2011; Storch et al., 2012; Unal et al., 2012; Wallukat and Schimke, 2014). These modes of activation may occur clinically, as in hypertension or preeclampsia (Wei et al., 2011; Zou et al., 2004). Candesartan and other inverse agonists may show increased therapeutic effects in these disease conditions (Wei et al., 2011; Zou et al., 2004). AT₁R signaling properties could be altered in pathology when AT₁R forms both homodimers and heterodimers (AbdAlla et al., 2004, 2000; Ayoub et al., 2015; Bellot et al., 2015; de Lourdes Gonzalez-Hernandez et al., 2010; Coupil et al., 2015; Martinez-Pinilla et al., 2015; Rozenfeld et al., 2011; Siddiquee et al., 2013; Wnorowski and Jozwiak, 2014) in disease states like atherosclerosis, preeclampsia, and chronic kidney disease (AbdAlla et al., 2001, 2004; de Lourdes Gonzalez-Hernandez et al., 2010).
β-arr2cent biased signaling in GPCRs is thought to be a relatively protective long-term effect of hormonal action. The discovery of β-arr2cent biased AT1R ligands preferentially activating the β-arr2cent-mediated signaling pathway (Violin et al., 2010; Wei et al., 2003) has gained much attention due to therapeutic potential in treating cardiovascular diseases (Boerrigter et al., 2011; Boerrigter et al., 2012). The size of the side chain of eighth residue present in different Ang II analogs determines the relative strength of AT1R signaling bias through G-protein and β-arr2cent pathways (Domazet et al., 2015; Rajagopal et al., 2011; Zimmerman et al., 2012). Ang II and other full agonists with Phe8 efficiently activate the Gq pathway. Whereas, Ile8 substitution in S118 changes it to a partial agonist for Gq signaling. It has been reported that strong β-arr2cent biased ligands (which functions through β-arr2cent while deficient in Gq-mediated functions) have smaller side chains (e.g., Ala) or even deletion of eighth residue (Rajagopal et al., 2011; Strachan et al., 2014).

Crystallographic Structures of AT1R

Crystallographic structures of both active and inactive conformational states are now available for AT1R, which provide insights into the conformational dynamics of the receptor in agonist and antagonist bound states.

Two inactive state structures were solved first, with an experimentally used antagonist ZD7155 (PDB ID: 4YAY) and clinically used inverse agonist olmesartan (PDB ID: 4ZUID) (Zhang et al., 2015a,b). For solving the structure, the human AT1R was engineered for thermal stability by truncating at the N-terminal region (delete Met1, Thr7-Asp16) and C-terminal tail at the end of helix 8, and by inserting a thermos stabilized apocytochrome, b562RIL (BRIL) at N-terminus. The final structure contains 289 out of 359 full-length residues of AT1R. However, these modifications did not alter the pharmacological and functional properties of the receptor for antagonist binding and signaling (Zhang et al., 2015b). High-accuracy computer model of hAT1R that included deleted portions were generated (Singh et al., 2018). The binding poses of experimentally used antagonists, and eight clinically used ARBs were found to be similar. Molecular dynamics simulation studies validated experimental Ki differences of ARBs as well as discriminatory mutagenesis data. Differences in spatiotemporal interactions of different ARBs was observed, which could account for efficacy differences reported for ARBs in various clinical trials. Nature and bonding energy contribution of some critical residues involved in binding all ARBs significantly differ. For example, Arg167ECL2 sidechain interacted with both imidazole and tetrazole groups in olmesartan but not in candesartan, losartan, and irbesartan. Instead, Lys199TM5 interacted with losartan and irbesartan. Valsartan interactions with Lys199TM5 is a weak water-mediated interaction. Both Arg167ECL2 and Lys199TM5 bond with two carboxylic groups of eprosartan, but mutating either of these residues do not affect eprosartan binding (Singh et al., 2018).

Initial structural papers also predicted that Ang II binding mode to different of ARBs, but sharing critical contact residues (Tyr3551.39, Trp842.60, Tyr872.63, Arg167ECL2, Lys1995.42, Ile2887.39, and Tyr2927.43) which can account for the competitive antagonistic relationship of ARBs with Ang II (Singh et al., 2018).

The active state structure of AT1R bound to the Ang II analog [Sar1, Ile8]Ang II (S1I8) was elucidated at 2.9 Å resolution (PDB ID: 6DO1) (Wingler et al., 2019a,b). The crystallization construct included all N-terminal residues and BRIL inserted into ICL3 as has been done for solving structures of many other GPCRs. In this configuration, the engineered receptor construct increased the affinity for Ang II ~2-fold and also displayed strong binding to an active-state conformation-specific AT1R nanobody, Nb. AT110i1. This study demonstrated unequivocally the critical residues of AT1R that bond with various sidechains in the peptide ligand and provided a structural interpretation for the movement of different segments of the receptor, which enable signaling. For instance, Pro7 and Ile8 of S1I8 (Phe8 in Ang II) interact with Trp842.60, Val1085.32, Leu1123.36, and Ile2887.39 which are located at the bottom of the orthosteric binding pocket. Ile8 of the peptide also forms a hydrophobic contact with His2566.51, a residue critical for AT1R activation (Noda et al., 1995). Unlike ARBs, the peptide ligand is involved in extracellular interactions with hydrophobic residues (Ile172ECL2, Tyr92ECL1, Val179ECL2, and Ala181ECL2) and charged residues (Asp2817.32 and Asp2636.58) confirming prior mutagenesis results (Feng et al., 1995; Fillion et al., 2013). The terminal carboxylate of the peptide interacts with Lys1995.42. This interaction has been observed as a key contact in AT1R activation by mutagenesis studies (Noda et al., 1995; Fillion et al., 2010). Due to inwards shifts of TM5 and TM7, and changes in ELC2 conformation, the ligand-binding pocket is substantially constricted around the peptide in the active state receptor compared to the inactive structure. The peptide-binding site of the AT1R showing S1I8-AngII binding residues of the receptor are shown in Fig. 3.

While comparing the active and inactive structures of AT1R, the most notable conformational changes were observed on the intracellular side of the receptor, as observed in almost all activated GPCR structures (Manglik and Kruse, 2017). Outward displacement of TM6 by 11 Å, along with the rotation of TM5 away from the G protein/β-arr2cent binding pocket and inward rotation TM7, was clearly observed. Additionally, a short 2 helix is formed by the reorganization of the ICL2, and a significant repositioning of helix 8 was also observed. Helix 8 in the inactive AT1R structure (Fig. 4) follows an atypical conformation bent away from the membrane plane. On the other hand, the active structure (Fig. 3) displays the conventional position of Helix 8 parallel to the membrane. The structural findings validate several functional studies suggesting that the Helix 8 of AT1R may be a critical motif for generating G protein independent signals.

The activation mechanism of AT1R suggested by Wingler et al. suggests the movement of Trp2537.48 and Tyr2927.43 to avoid a steric clash with Phe8 sidechain of Ang II, resulting ultimately in the breakage of a hydrogen bond between Asn1111.35-Asn2957.46 present in the inactive structure (Wingler et al., 2019a,b). A slightly different scheme for activation is suggested by MD simluation studies. This model suggests that Phe8 in Ang II is involved in a van der Waals “grasp” interaction with Ile2887.39 in AT1R. An induced mechanical strain pulls Tyr2927.43 and breaks critical inter-helical H-bonds, first between Tyr2927.43 and Val1085.32 and
Fig. 3  The peptide-binding site of the AT$_1$R showing S1I8-AngII binding residues of the receptor and position of helix 8 in an active state of AT$_1$R. Sarcosine1, Isoleucine8-AngII is abbreviated as S1I8-AngII. The S1I8-AngII-binding residues of AT$_1$R are shown in green. S1I8-AngII is shown in orange.

Fig. 4  The ARB’s binding site of the AT$_1$R showing ZD7155 (antagonist) binding residues of the receptor and position of helix 8 in an inactive state of AT$_1$R. The antagonist ZD7155 binding residues of AT$_1$R are shown in orange. ZD7155 is shown in violet.
second between Asn1113.35 and Asn2957.46 (Singh et al., 2019). The consensus step in both schemes, breakage of hydrogen bond(s) between Asn1113.35–Asn2957.46 was shown to activate AT1R ligand independently by mutagenesis studies (Noda et al., 1995; Singh et al., 2019; Wingler et al., 2019b).

Approved ARBs and Their Clinical Potential

Eight ARBs, azilsartan, candesartan, eprosartan, olmesartan, irbesartan, losartan, telmisartan, and valsartan are in clinical use as safe drugs primarily to control blood pressure. These drugs vary in their duration of action and efficacy when repurposed in the treatment of disorders of kidney, heart, lungs, fibrogenesis of liver, kidney, or heart and stroke prevention. Additional indications have been recommended. For example, telmisartan can improve glucose metabolism and lipid profile (Nedogoda et al., 2013). Losartan shows uricosuric activity (Nedogoda, 2011). Reduction in inflammatory processes in vessel walls was observed in patients with arterial hypertension and metabolic syndrome when treated with ARBs, thus decreasing their risk for cardiovascular disease and development of diabetes mellitus (Savoia and Schiffrin, 2007). Based on the experimental data, sartans are reported to have possible therapeutic effects in the treatment of autoimmune diseases such as rheumatoid arthritis (Silveira et al., 2013) and multiple sclerosis (Lanz et al., 2010).

AT1 receptors are abundantly expressed on the endothelial cells of the blood-brain barrier, and these have been benefited by the neuroprotective actions of the ARBs (Saavedra et al., 2011). ACE inhibitors captopril and perindopril, and ARB’s losartan, telmisartan, and candesartan have shown neuroprotective effects in animal Parkinson’s disease models. These effects seem to be mediated by a decrease in the overproduction of reactive oxygen species (ROS) (Perez-Lloret et al., 2017). Preclinical and clinical data support potential antidepressant properties of ACEIs and ARBs. For example, captopril (Costall et al., 1990), losartan (Llano Lopez et al., 2012; Srinivasan et al., 2003), valsartan (Ping et al., 2014), irbesartan (Ayyub et al., 2017), telmisartan (Aswar et al., 2017), and Candesartan (Benicky et al., 2011; Saavedra et al., 2006) had positive effects on depression and reduced anxiety behavior, whereas other antihypertensive agents did not (Vian et al., 2017).

Dual-Acting Angiotensin-Receptor/NEP Inhibitor (ARNI)

Sacubitril/valsartan or LCZ696, called Entresto (developed by Novartis), is a novel dual-acting drug formulation comprising equimolar amounts of the ARB valsartan and the nephrilysin inhibitor sacubitril (Sacubitril is a prodrug that is activated to sacubitrilat by de-ethylation via esterases). This drug combination increases nephrilysin activity while inhibiting the harmful effects of the RAS, without affecting bradykinin and other nephrilysin-derived vasoprotective factors. Sacubitril/valsartan has demonstrated clinical efficacy in lowering blood pressure in patients with primary hypertension and patients with or without heart failure. The landmark clinical trial named PARADIGM-HF [Prospective comparison of ARNI (LCZ696) with ACEI (Enalapril) to Determine Impact on Global Mortality and morbidity in chronic Heart Failure] showed that LCZ696 was significantly more effective than enalapril in treating heart failure (McMurray et al., 2014; Mogensen et al., 2018). Sacubitril/valsartan was approved by the FDA in 2015. This drug is now included in European (Ponikowski et al., 2016) and American (Yancy et al., 2016) clinical practice guidelines for the treatment of heart failure.

Counter-Regulatory RAS With its Importance and Limitations

Counter- regulatory or the non-canonical or the non-classical RAS primarily consists of Ang II/Ang III-AT2R, and the ACE2-Ang-(1–7)-MasR axis. Generally, it is believed that the counter-regulatory RAS functions in a manner antagonistic to the deleterious effects of a dysregulated classical RAS, the Ang II-AT1R axis. This includes actions of extensively studied ligand/receptor actions such as Ang (1–7)/MasR and Ang II or Ang III/AT1R, as described earlier (Forrester et al., 2018). There are also reports suggesting the actions of Ang (1–7)/AT1R on the cardiovascular system (Mendoza-Torres et al., 2018). Potential roles of ligands, alamandine, and Angiotensin A remain poorly characterized. Ang-(1–7) has also been suggested to function as a β-arrestin-biased agonist of AT1R (Teixeira et al., 2017). Alamandine functions through MRGD and activates the AMP-activated protein kinase (AMPK)–nitric oxide (NO) pathway, which prevents Ang II-induced hypertrophy (Jesus et al., 2018). Angiotensin 1–5 has been shown to induce atrial natriuretic peptide (ANP) secretion through the Mas receptor and activating the PI3K–Akt–endothelial NO synthase pathway (Yu et al., 2016).

Different research groups have different opinions about whether Ang-(1–7) binds directly to the Mas receptor or not, and this has been reflected from the contradictory results published. Some showed that Ang-(1–7) can oppose the Ang II signaling as a result of heterodimerization between AT1R and Mas receptor but not by direct binding to the Mas receptor (Gaidarov et al., 2018; Kostenis et al., 2005). However, others demonstrated the binding of radiolabeled or fluorescent Ang-(1–7) to the Mas receptor-expressing tissue (Santos et al., 2003). In summary, more research is required to confirm whether Ang-(1–7) acts as an endogenous agonist of the Mas receptor.
Crystal Structures of AT2R

AT2R structures have been solved with either small-molecule ligands (quinazolinone-biphenyl tetrazole derivatives 1 and 2) named as compound 1 and compound 2 (Zhang et al., 2017b). The compound 1 is AT2R selective, while compound 2 is AT1R/AT2R dual ligand [PDB ID: 5UNF; 5UNG; 5UNH]. Following this study, the peptide ligand-bound AT2R structures were solved with S1I8 (PDB ID: 5XJM) or the endogenous peptide, Ang II (PDB ID: 6JOD) (Asada et al., 2018, 2020).

A nearly similar AT2R construct was used to obtain both S1I8-Ang II-bound and Ang II bound crystal structures. The AT2R-specific antibody, Fab4A03, was added during the purification of both the structures to increase thermal stability and facilitate crystallization. Both structures lack the putative C-terminal palmitoylation site (residue range: 35–346) and N-terminal glycosylation sites. The difference lies in the type and position of BRIL that was inserted in the third intracellular loop in S1I8 bound AT2R and fused at N-terminus in Ang II-bound AT2R. The S1I8-Ang II bound AT2R sequence includes 35–346 of the 363 residues, while the Ang II bound AT2R sequence includes 1–346 of 363 residues.

The structures demonstrated AT2R to accommodate both peptides in a very similar binding mode. The Ang II sidechains interacted with AT2R residues whose position and chemical characteristics are conserved, as seen in the AT1R structure. Arg2 forms salt bridges with Asp2796.58 and Asp2977.32, which are conserved as Asp2636.58 and Asp2817.32 in AT1R. The guanidinium group of Arg182ECL2, which is conserved as Arg167ECL2 in AT1R bonds with carbonyl oxygens of His6 and Pro7 in Ang II. Phe8C-terminal carboxyl group forms a salt bridge with the side chain of Lys2155.42, which is conserved as Lys1995.42 in AT1R. Phe8 of Ang II also interacts with Leu1243.32, Met1283.36, Trp2696.48, Phe2726.51, and Phe3087.43. Ligand binding and mutagenesis experiments proved that Tyr1042.64, Met1283.36, Lys2155.42, Trp2696.48, Phe2726.51, Arg182ECL2, Asp2977.32, and Phe3087.43 play vital roles in Ang II binding. Although Fab4A03, AT2R specific antibody used during crystallization, does not affect the conformation of the side chains critical for ligand binding in both Ang II-bound and S1I8-Ang II-bound AT2R, ECL2 conformation on the surface is affected in both structures. The peptide-binding site of the AT2R with Ang II binding residues of the receptor are shown in Fig. 5.

The residues at the bottom of the ligand-binding pocket and Met1283.36 (Leu1123.36 for AT1R), seems to play a key role in AT2R activation. Upon Ang II binding, Met1283.36 moves toward Phe3087.43 (Phe3087.43 for AT1R) to make room for Phe8 side chain of Ang II, leading to the rotation of TM7 possessing Phe3087.43. These residues form hydrophobic core at the bottom of the ligand-binding cavity of AT2R, as observed in AT1R. The insertion of Ang II Phe8 residue into this hydrophobic core appears to trigger the activation of the receptor, where the conformational change is transferred through the region containing the internal lock, Asn1113.35, Asn2957.46 leads to the breakage of the hydrogen bond between these residues during AT1R activation. In AT2R, Ser3117.46 is present in place of Asn2957.46, and a hydrogen bond between Asn1273.35 and Ser3117.46 is not observed in the Ang...
II-bound AT2R structure. It is not clear whether these residues form hydrogen bond in the inactive conformation since AT2R inactive structure is not available. However, AT2R has been described as a constitutively active receptor (Miura and Karnik, 2000), therefore it may naturally lack the hydrogen bond between Asn1273.35 and Ser3117.46.

In support of this view, an active like conformation of AT2R was captured (Asada et al., 2018, 2020; Zhang et al., 2017b). Structures showed that AT2R lacks the key conformational locks seen in AT1R. 1) TM5/TM6 phenylalanine cluster (Phe2085.51/Phe2496.44/Phe2506.45) in AT1R are replaced by aliphatic residues (Leu2245.51/Phe2656.44/Ile2666.45) in AT2R. In all the three crystal structures available for AT2R (mentioned under structure of AT2R section), these residues align with active AT1R structure rather than inactive AT1R. 2) The Asn1113.35-Asn2957.46 hydrogen bond in the AT1R inactive state is not present in the AT2R structure. Instead, AT2R has Ser3117.46 (in place of Asn2957.46), and Asn3.35 has moved away from the receptor core. 3) In AT1R, T292F mutation increases the affinity towards Ang II while decreases towards antagonists. Whereas Tyr2927.43 is substituted by Phe3087.45 in the AT2R, denoting a mutation-induced-stabilization of an active-like state. 4) Finally, AT1R shows highly unusual behavior as it does not seem to signal through traditional G-protein- and β-arrestin-mediated signaling pathways, and this is potentially due to helix 8 overlap over the transducer-binding site of AT2R (Zhang et al., 2017a).

The MasR and Mas-related GPCRs exhibit structural features of AT1R that cause constitutive activation. In signaling experiments, high constitutive signaling by MasR is reported. Therefore intrinsic constitutive activation is likely a feature of these receptors. However, there has been no structural investigations focused on MasR and its activation by ligands such as Ang (1 – 7) or related peptides.

Targeting the Counter-regulatory Axis of RAS and Novel Therapeutic Approaches

AT1R signaling and functions are better understood now and being translated to develop potential drugs targeting AT1R. The developmental drugs are AT1R agonists with primary indications for use in fibrotic diseases and diabetic nephropathy. A non-peptide AT1R agonist, Compound 21 (C21) (Fig. 6), has successfully completed phase I clinical testing and entered Phase II clinical study in patients with Pulmonary fibrosis in systemic sclerosis (SSc) and Idiopathic pulmonary fibrosis (IPF). In addition, a phase II study of C21 in patients with COVID-19 proposed. The rationale behind this study is to address an imbalance in the local RAS caused by ACE2 inactivation due to COVID-19 binding. C21 may suppress inflammatory mediators by acting directly on the AT1R and bypass the way by which the virus disables the system (See Relevant Websites Section). Selective AT2R agonist non-peptide Compound 21 (C21) (Miura and Karnik, 2000) is currently being tested in a phase I clinical trial with a focus on oncology indications. The AT2R antagonist RB150/QGC001 (4,4-dithio-{bis[(3S)-3-aminobutyl sulfonic acid]}) has been described as a constitutively active receptor (Miura and Karnik, 2000), therefore it may naturally lack the hydrogen bond between Asn1273.35 and Ser3117.46.

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RAS Components as Targets of New Diseases Including COVID-19

RAS is a complex, multifunctional system that possesses important roles beyond the cardiovascular system. Alterations in the expression of these components were shown to be involved in various diseases. For example, brain RAS involving the ACE2/Ang-(1 – 7)/Mas receptor axis and the Ang IV/insulin-regulated aminopeptidase pathways may play a role in Parkinson’s and Alzheimer’s diseases (Wright et al., 2013). ACE, Ang-(1 – 7), AT2 receptors, and N-acetyl-Ser-Asp-Lys-Pro may have a role in
hematopoiesis (Rodgers and Dizerega, 2013). Aldosterone produced locally may have a pathogenic role (Aroor et al., 2013; De Mello and Frohlich, 2014), and the ACE2/Ang-(1–7)/Mas receptor pathway may take part in reproduction, fetal programming, and cancer (Chappell et al., 2014; Herr et al., 2013). Activation of skeletal RAS plays a major role in bone diseases, such as arthritis, osteoporosis, and deterioration, as well as in fracture healing (Zhao et al., 2019).

The recently emerged disease involving the RAS component is COVID-19 (Coronavirus Disease 2019) pandemic. The virus is named SARS-CoV-2 (severe acute respiratory syndrome coronavirus-2). The virus binds to the ACE2 receptor for entry into target cells. ACE2 prevents adverse effects of Ang II by degrading it to Ang-(1–7) (Donoghue et al., 2000; Hamming et al., 2007) and has been implicated in hypertension (Allred et al., 2002; Crackower et al., 2002), diabetes (Tikellis et al., 2003), Ang-(1–7) regulation during pregnancy (Brownihan et al., 2003), heart failure and ventricular remodeling (Donoghue et al., 2003; Zisman et al., 2003). ACE2 predominantly expressed on epithelial cells of lungs, heart, kidney, and intestine (Donoghue et al., 2000; Zhang et al., 2020; Zhao et al., 2020) is a functional receptor for the coronavirus (Li et al., 2003). Interaction between the SARS-CoV-2 spike receptor-binding domain (RBD) and ACE2 has been shown in atomic-resolution by structural studies (Lan et al., 2020; Wang et al., 2020). These findings shed light on virus recognition, infection, and provide important structural information about the development of therapeutic treatment against this emerging virus.

Several comorbidities, including cardiovascular diseases, diabetes mellitus, and hypertension, are involved in COVID-19 patients with a severe course of progression and contributing to higher mortality risk (The Novel Coronavirus Pneumonia Emergency Response Epidemiology Team, china, 2020). Some research groups reported that both ACEI and ARBs could
substantially increase the mRNA expression of cardiac ACE2 (Ferrario et al., 2005), which may increase virus susceptibility. However, due to lack of evidence about the potential negative impact of these medications on COVID-19 infection, European and American Societies of Cardiology expressed that ACEIs and ARBs are safe to continue and should be prescribed according to established guidelines (Sommerstein et al., 2020). In conclusion, cardiovascular diseases and/or their therapy, by influencing ACE2 levels, may play a crucial role in infectiveness and outcome of COVID-19. Whether treatment or disease triggered upregulation of ACE2 affects the course of COVID-19 needs to be determined.

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