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Breaking the Enigma Code of Angiotensin II Type 2 Receptor Signaling

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In this issue of *Structure*, Asada et al. (2019) present angiotensin receptor AT_2R structure in complex with its main endogenous agonist, AngII peptide. Complementing the previous structural studies, the new complex structure sheds light on the AT_2R activation mechanism and opens new avenues for drug discovery targeting this enigmatic receptor.

Renin-angiotensin system (RAS), controlled by angiotensin II octapeptide signaling at angiotensin AT_1R and AT_2R receptors, plays a key role in regulating cardiovascular and electrolyte homeostasis. The more recently discovered "protective arm" of RAS, which involves several other angiotensin peptide isomers and signals largely via AT_2R (as well as proto-oncogene MAS receptor), has also been implicated in a variety of tissue-protective functions in the human body. While AT_1R has a well-understood canonical G protein-coupled receptor (GPCR) signaling via G protein and arrestin and is a prominent target of blockbuster antihypertensive drugs—angiotensin receptor blockers (ARBs), also known as "Sartans" (Zaman et al., 2002)—the AT_2R sibling has been notorious as an "enigmatic" receptor (Porrello et al., 2009).

During the 25 years since its discovery, AT_2R has been implicated in numerous physiological functions with great potential for drug discovery (Paz Ocaranza et al., 2020). AT_2R activation by AngII is believed to directly oppose AT_1R signaling, mediating vasodilatory effects and reducing blood pressure. In addition, many studies show that AT_2R activation by Ang(1-9) and, potentially by Ang(1-7), confers protective, antifibrotic, and anti-inflammatory properties in a variety of tissues including the heart, lung, kidney, and brain (Paz Ocaranza et al., 2020). Intriguingly, these protective peptides are produced by angiotensin converting enzyme 2 (ACE2), which is exploited as a host factor by SARS and COVID-19 viruses, aggravating lung tissue damage often caused by these dangerous coronavirus infections. While AT_2R is more abundant in the fetus and neonate, where it is believed to be involved in vasculature growth and neural development, the receptor retains expression in several adult tissues and is greatly induced in tissues undergoing repair. The initial interest in the protective properties of AT_2R arose from clinical studies showing that ARBs can be superior to other antihypertensive drugs in the prevention of stroke, even with no differences in blood pressure, and that one of the protective mechanisms was mediated by AT_2R (Thöne-Reineke et al., 2006). As such, AT_2R-selective ligands, e.g., Ang(1-9) peptide or small molecule agonist C21, are being extensively tested in preclinical studies for acute lung injury and fibrosis, kidney fibrosis, Alzheimer's disease, and stroke. Notably, AT_2R antagonism in the peripheral nervous system also shows promise in potential clinical applications as a treatment for chronic neuropathic pain. Small molecule antagonist EMA401 showed efficacy in treating neuropathic pain in phase II clinical studies (Rice et al., 2014), until the development of this candidate was stopped due to off-target side effects.

On the other hand, translation of these very promising findings into clinical therapies has been hampered by very

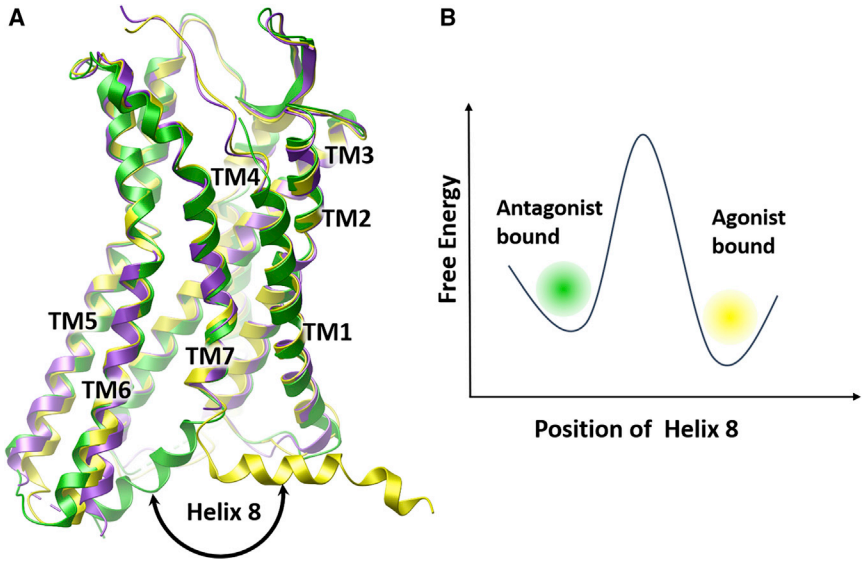

Figure 1. Comparison of AT_2R Conformers
(A) Superimposition of crystal structures in complex with antagonist (compound 1, PDB: 5UNF, green ribbon), partial agonist ([Sar1,Ile8]-Ang II, PDB: 5XJM, magenta ribbon), and full agonist (AngII, PDB: 6JOD, yellow ribbon).
(B) Hypothetical energy profile for the conformational change in helix 8.

puzzling functional mechanisms ascribed to AT_2R activation on the molecular and cellular levels, as well as by the very limited toolchest of AT_2R-selective drug-like compounds. AT_2R is believed to bypass canonical G protein- and arrestin-mediated signaling, activating instead downstream effectors such as ERK1/2 and NOS (Porrello et al., 2009). Several other downstream pathways have been suggested, but the mechanistic understanding of AT_2R signaling is still far from obvious. Moreover, on the cellular level, a recent discovery implicates AT_2R expressed in macrophages, but not in neurons themselves, in neuropathic pain induced by locally released AngII, adding a new twist to the development of AT_2R-mediated pain remedies (Shepherd et al., 2018).

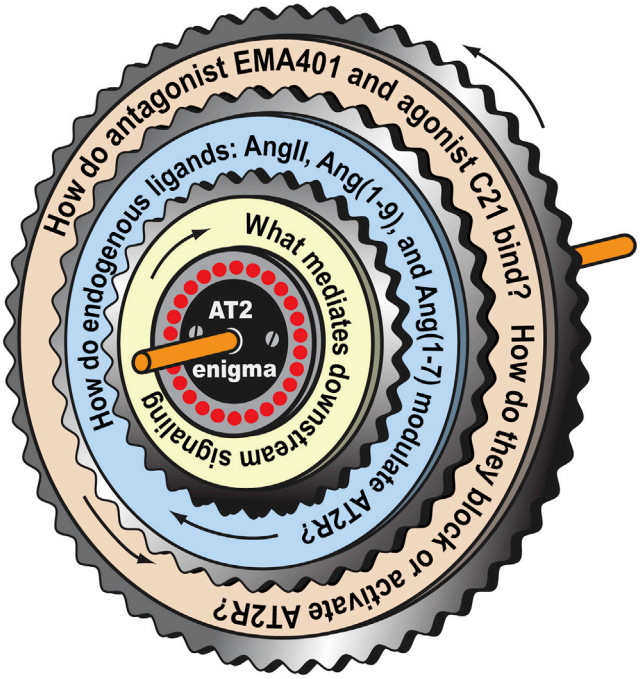

Figure 2. Layers of AT_2R Signaling Enigma
Several questions at physiological, molecular, and pharmacological levels remain to be answered to break the enigma code of AT_2R function.

When the first structure of AT_2R was solved in 2017 (Zhang et al., 2017), the receptor definitely lived up to its "enigmatic" reputation. Most strikingly, the antagonist-bound structures of AT_2R consistently showed all the hallmarks of GPCR active state, including all microswitches and helical rearrangements on the intracellular side of the receptor, including an outward shift of TM6 and inward shift of TM7 (Katritch et al., 2013). This conformational arrangement was the same for different antagonists and different crystal packing of AT_2R and drastically differed from its sibling AT_1R, which shows a canonical inactive state with antagonists and canonical active state with agonists.

Moreover, this antagonist-bound "active state" in AT_2R came with a catch—helix 8, instead of sticking to the intracellular surface of the membrane as in other GPCRs, is switched to a previously unknown position, bundling to helices TM5 and TM6 and thus hindering possible interactions of AT_2R with G protein or arrestin. This position of helix 8 was corroborated by strong interaction interface with TM5/TM6, as well as with molecular dynamics simulations showing that even when pulled slightly away, helix 8 returned to its blocking position. Furthermore, the AT_2R structure with partial agonist derivative of AngII, [Sar1, Ile8]-AngII, was also found in a similar active-like state (Asada et al., 2018), though completely lacking electron density for helix 8 and thus suggesting high conformational flexibility of this helix.

In this issue of *Structure*, the structure of AT_2R complex with endogenous AngII peptide and a stabilizing FAB antibody from the So Iwata group reveals a number of intriguing details and insights into the receptor workings (Asada et al., 2019). In general, it shows the same overall active-state conformation as in previous complexes with small molecule antagonists (Zhang et al., 2017) and the partial agonist analog of AngII (Asada et al., 2018) (Figure 1). But it also gives insight into what differentiates the full agonist binding in terms of receptor conformation. First of all, the differences at the bottom of the ligand-binding pocket are notable. The terminal Phe8 side chain of AngII (but not Ile8 of the partial agonist or the small molecule antagonist) is pushing Met128 side chain to change conformation where it impinges on Trp269, one of the key residues in the activation mechanism. This reshaped binding pocket may provide a better template for structure-based design of full AT_2R agonists, though small molecules like C21 may further adjust the orthosteric pocket.

The most intriguing, though, is the conformation of helix 8 resolved in this agonist-bound structure. Unlike the antagonist-bound conformation, where helix 8 was blocking the intracellular site of G protein binding, or partial agonist structure, where helix 8 was completely unresolved, the new agonist structure reveals helix 8 in the canonical membrane-bound position, where it does not obstruct the intracellular pocket. These observations and comparative analysis of the structure suggest that the AT_2R resting state naturally prefers active-like conformation, but with downstream signaling blocked by helix 8; partial agonists partially unblock it by destabilizing the TM6-bound conformation of helix 8, while full agonists further stabilize helix 8 in the membrane-bound state, allowing full downstream signaling. One has to be cautious in jumping to conclusions here, though, as noted by Asada et al., and further studies are needed to confirm that this helix 8 observation is not an artifact of crystal packing.

There are at least three layers of the puzzle to break the enigmatic code of AT_2R function, which remain to be resolved by further structural, biochemical, and computational studies (Figure 2):

(1) On the molecular level, if AT_2R signaling is not G protein and β arrestin mediated, what are the actual cognate proteins that recognize AT_2R active state, and how are they connected to the downstream signaling via ERK1/2 and NOS?
(2) On the physiological level, what is the functional and physiological difference between the three known endogenous AT_2R ligands: AngII, Ang(1-9), and Ang(1-7)? Do they confer similar signaling

profiles or are they differentially biased to some downstream pathways against the others? How does AT_2R signaling interact with other RAS pathways?

(3) On the pharmacological level, how do small molecule drug candidates like agonist C21 and antagonist EMA401 bind AT_2R, inducing activation or blockade, and how can the structural information on these ligands be used to rationally design new, more potent and selective agonists and antagonists?

The answers to such questions require multidisciplinary studies including further structural and molecular dynamics analysis of AT_2R complexes, expansion of the repertoire of AT_2R-selective agonists and antagonists, and thorough characterization of their function. The rewards to breaking the AT_2R enigma code will be high, as it would greatly facilitate the discovery of new AT_2R-mediated therapies for major chronic conditions like neuropathic pain, stroke, Alzheimer's disease, and cancer.

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