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A novel mechanism of action for angiotensin-(1-7) via the angiotensin type 1 receptor

Running title: Ang-(1-7) is a biased AT₁R agonist

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Angiotensin-(1-7) [Ang-(1-7)] was originally thought to be an inactive metabolite of angiotensin II (Ang II) in the renin angiotensin system (RAS). However, reported biological effects and seminal discoveries of an angiotensin converting enzyme (ACE) homologue, ACE2 which metabolises Ang II to Ang-(1-7),\(^1\) combined with identification of the G protein coupled receptor Mas as an endogenous Ang-(1-7) receptor\(^2\) defined a natural counter-regulatory ACE2/Ang-(1-7)/Mas axis of the RAS. The counter-regulatory axis of the RAS is firmly established to inhibit detrimental effects mediated through the classical ACE/ Ang II/angiotensin type 1 receptor (AT\(_1\)R) axis. Moreover, it is simplistic to separate the RAS into these two axes as the systemic and local tissue-specific RAS includes a wide range of molecules with biological action, including the alternative Ang II receptor, the angiotensin type 2 receptor (AT\(_2\)R), other peptide metabolites, e.g. Ang-(1-9), Ang III and Ang IV, and recent discoveries including an Ang-(1-7) metabolite, Alamandine, and its receptor Mas-related G protein-coupled receptor D (MrgD).\(^3\)

Ang-(1-7) mediates varied physiological and therapeutic effects via Mas, including preventing adverse tissue remodelling, improving vascular and cardiac function and promoting wound healing. Importantly, effects of Ang-(1-7) are not all directly through antagonising the ACE/Ang II/AT\(_1\)R axis. In fact Ang-(1-7), through Mas and G\(_\text{ias}\),\(^4\) activates distinct intracellular signalling leading to production of nitric oxide, modulation of ERK signalling and stimulation of cAMP release [reviewed in\(^5\)]. Therefore, Ang-(1-7) is well established as an independent RAS component and is being explored clinically, e.g. for stimulating haematopoietic recovery in cancer patients following chemotherapy.\(^6\)

In this issue of Hypertension the complexity of RAS interactions, and particularly Ang-(1-7) function, is further expanded by Galandrin et al, via a series of elegant molecular pharmacology studies that decipher a novel role for Ang-(1-7) as a biased AT\(_1\)R agonist\(^7\) and
therefore define a previously unrecognised mechanism of action. The concept of biased agonism at the AT₁R is relatively new and stems from the recognition that biased AT₁R agonists, e.g. the peptide [Sar¹, Ile⁴, Ile⁸]-angiotensin II, stimulate β-arrestin recruitment to the receptor leading to AT₁R internalisation, β-arrestin-dependent signalling and potential therapeutic outcomes which are distinct to the classical G protein-coupled responses.⁸ Galandrin et al performed a systematic evaluation of angiotensin peptides was performed in AT₁R-overexpressing HEK293T cells.⁷ Bioluminescence resonance energy transfer (BRET) was used to assess abilities of Ang II, Ang III, Ang IV and Ang-(1-7) to activate classical Gα signalling. While Ang III and AngIV mimicked Ang II in activating Gα signalling via Gα₁α₁₀, Gαq/11 and Gα₁₃ families, Ang-(1-7) did not. Binding affinity studies revealed all angiotensin peptides displaced ¹²⁵I-Ang II from the AT₁R, with affinities from nM (Ang II and Ang III) to μM (Ang IV). Ang-(1-7) was reported to have a Ki of 360 nM and act as a natural neutral antagonist at the AT₁R by shifting the Ang II response curve to the right for activation of Gα₁₃ and, with lower potency, Gαq. Importantly, Ang-(1-7) also acted as a biased AT₁R agonist by stimulating β-arrestin2 recruitment. The potency of Ang-(1-7) was lower than Ang II, but in line with its binding affinity and inhibited by the AT₁R blocker candesartan.

Biased AT₁R agonism is being actively investigated for therapeutic applications. The classical actions of Ang II at the AT₁R in hypertension and cardiovascular disease (CVD) are mainly mediated through activating Gαq. Conversely, AT₁R signalling also leads to non-G protein-mediated signalling through β-arrestin2 which is therapeutic in CVD. Currently, the β-arrestin2-biased AT₁R molecule TRV1200027 is being explored in clinical trials for heart failure after demonstrating efficacy in reducing blood pressure and improving cardiac function in rodent models.⁹ The therapeutic development of biased AT₁R agonists is important in the context of Ang-(1-7) as a biased AT₁R agonist. Galendrin et al moved from
in vitro molecular pharmacology studies to a whole organ model in isolated aortas from wild type and AT\textsubscript{1}R knockout mice.\textsuperscript{7} Ang-(1-7) inhibited phenylephrine-mediated contraction in wild type, but not AT\textsubscript{1}R knockout aortas. Moreover, effects of Ang-(1-7) in wild type aorta were not inhibited by the mas antagonist A-779, nor the AT\textsubscript{2}R antagonist PD-123,319, but were by candesartan. Intriguingly, when Ang II was utilised as a vasoconstrictor in aortas from wild type mice, co-application of Ang-(1-7) potentiated the effect in a manner sensitive to the Mas antagonist A-779. The reason for this finding is not clear but highlights the complexity of RAS interactions. Previous studies have highlighted cross-talk between Ang-(1-7) and other RAS receptors, with effects reported via the AT\textsubscript{2}R\textsuperscript{10} and MrgD.\textsuperscript{4} However, AT\textsubscript{2}R studies have predominantly utilised the AT\textsubscript{2}R antagonist PD-123,319 which is also reported to inhibit MrgD which may complicate interpretation.\textsuperscript{3, 4} Furthermore, Mas is also a physiological antagonist of the AT\textsubscript{1}R, again highlighting the complexity of cross-talk in the RAS.\textsuperscript{11} These new studies\textsuperscript{7} are the first demonstration that Ang-(1-7) can both interact with the AT\textsubscript{1}R and mediate distinct signalling effects and provide new knowledge regarding how RAS components interact via crosstalk.

What remains to be dissected is how these individual peptide/receptor interactions function in vivo. In a diseased tissue there are likely to be differing levels of RAS-related receptors and dynamically changing angiotensin peptide levels and it will be difficult to determine the relative contribution of individual peptides acting at different receptors. Nevertheless it will be important to research the contribution of biased agonism from Ang-(1-7) at the AT\textsubscript{1}R and integrate the knowledge with that known through Mas agonism.

In summary, this study\textsuperscript{7} has defined novel interactions in the RAS and contributes to increased knowledge of how RAS peptides and receptors intersect to mediate biological outcomes. Further understanding of these aspects of the RAS may lead to the next generation
of RAS-targeting medicines capable of integrating differential signalling outcomes into synergistic action in CVD.

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None.
References


type 1a receptor in isolated cardiac myocytes. *Proc Natl Acad Sci U S A.* 2006;103:16284-16289.


**Figure Legend**

Figure 1. New model of angiotensin-(1-7) action. Angiotensin II (Ang II) acts at the angiotensin type 1 receptor (AT\(_1\)R) and stimulates (patho)physiological G protein signalling in cardiovascular disease, mainly through the G\(\alpha_q/i\) family. Ang II also activates G protein-independent signalling via recruitment of \(\beta\)-Arrestin2 (\(\beta\)Arr2) leading to therapeutic effects. The counter-regulatory renin angiotensin system axis peptide angiotensin-(1-7) [Ang-(1-7)] mainly acts at Mas to stimulate diverse therapeutic effects in different diseases via G\(\alpha_s\) activation. Ang-(1-7) also binds the AT\(_1\)R leading to \(\beta\)Arr2 recruitment and vasorelaxation in isolated aortas.
Ang II

\[ \text{Ang II} \rightarrow \text{AT}_1R \rightarrow G_{uR} \]

Pathological effects
- Hypertension
- Cardiac hypertrophy
- Pro-fibrotic
- Pro-proliferative

Ang-(1-7)

\[ \text{Ang-(1-7)} \rightarrow \text{Mas} \rightarrow G_{oR} \]

Therapeutic effects
- Reduced BP
- Vasorelaxation
- Increased cardiac performance

Therapeutic effects
- Reduced BP
- Vasodilation
- Increased cardiac output
- Anti-fibrotic
- Anti-proliferative