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Insight into the role of urotensin II-related peptide tyrosine residue in UT activation

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ABSTRACT

While sharing common biological activity, the two endogenous ligands of the G protein-coupled receptor UT, e.g. urotensin II (UII) and urotensin II-related peptide (URP), also exhibit distinct effects that could be explained by distinct interactions with their cognate receptor (UT). Accordingly, introduction of a similar substitution at the intracyclic Tyr residue in UII and URP led to compounds with divergent pharmacologic profiles. Hypothesizing that the Tyr^6 residue of URP is a key-element to understand the specific activation of UT by URP, we undertook a study of the structure-activity relationship in which this particular residue was replaced by non-natural and constrained amino acids. Each compound was evaluated for its ability to bind UT, to induce rat aortic ring contraction and to activate Gq and G_{12} signaling pathways. We identified [Pep^6]URP, that binds UT with an affinity similar to that of URP, but behaves as a biased ligand. Used as an antagonist, this peptide is also able to selectively reduce the maximal aortic contraction of URP but not UII. Our results suggest that the orientation of the Tyr residue can stabilize at least two different conformations of UT, leading to biased signaling and a probe-dependent allosteric effect.

1. Introduction

The urotensinergic system, formed by a G protein-coupled receptor (GPCR) termed UT and two endogenous peptide ligands, Urotensin II (UII, H-Glu-Thr-Pro-Asp-[Cys-Phe-Trp-Lys-Tyr-Cys]-Val-OH) and Urotensin II-related peptide (URP, H-Ala-[Cys-Phe-Trp-Lys-Tyr-Cys]-Val-OH), is currently regarded as a potential key contributor to cardiovascular function [1,2]. Notably, UII is considered as one of the most potent endogenous vasoconstrictor and inotropic agents ever described [3,4]. Over the years, evidence has highlighted the involvement of this system in the genesis and progression of various cardiovascular pathologies including atherosclerosis [5] and pulmonary arterial hypertension (PAH) [6]. Multiple animal studies have shown the therapeutic potential of UT antagonists for treatment of such pathologies but also hypertension, metabolic syndrome, as well as cardiac and renal failure [7–11]. However, discrepancies observed with urantide, an antagonist/partial agonist of the urotensinergic system [12], and other candidates as well, point out a greater need for understanding UT pharmacology at both the molecular and cellular levels.

UII and URP are cyclic peptides that share a common and strictly conserved bioactive cyclic core (-Cys-Phe-Trp-Lys-Tyr-Cys-) but differ by their extracyclic N-terminal residues [4]. Accordingly, while modifications at the N-terminus of UII or URP have almost no impact on their biological activity, several structure-activity relationship analyses, including Ala- and D-scans, revealed that both the nature and orientation of their intracyclic residues were crucial for UT recognition and activation [13–18]. In particular, these studies demonstrated the important role played by the tyrosine residue in UII and URP. For instance, and as recently reported, the replacement of the Tyr residue with more hydrophobic and/or bulky residues such as (3,4-Cl)Phe or 3-iodo-

Abbreviations: BRET, bioluminescence resonance energy transfer; ACN, acetonitrile; Bip, biphenylalanine; Boc, tert-butoxycarbonyle; BOP, (Benzotriazol-1-yloxy) tris(dimethylamino) phosphonium hexafluorophosphate; CHO cells, Chinese Hamster Ovary cells; DCM, dichloromethane; DIEA, N,N-diisopropylethylamine; DMEM, Dulbecco's Modified Eagle's medium; DMF, dimethylformamide; FBS, foetal bovine serum; Fmoc, Fluorenylmethyloxycarbamate; GFP 10, green fluorescent protein 10; GPCR, G protein-coupled receptor; HEK 293 cells, Human Embryonic Kidney 293 cells; $K_3[Fe(CN)_6]$, potassium ferricyanide; MALDI-TOF, Matrix-assisted laser desorption/ionization-Time of flight; NMR, nuclear magnetic resonance; PAH, pulmonary arterial hypertension; $PdCl_2(PPh_3)_2$, Bis(triphenylphosphine)palladium (II) dichloride; Pep, (phenylethynyl)-phenylalanine; Phe(pI), para-iodo-phenylalanine; Phg, phenylglycine; RlucII, *Renilla* luciferase II; RP-HPLC, Reverse phase-high performance liquid chromatography; Tic(7-OH), 7-hydroxy-1,2,3,4-tetrahydroisoquinoline; Tyr(mI), meta-iodo-tyrosine; UII, Urotensin II; URP, Urotensin II-related peptide; UT, urotensin II receptors.

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Tyr as well as the conservation of a specific g⁻ orientation of the side chain of these residues gave rise to potent UT agonists [19]. While these assumptions are true for UII and its equiactive analog $UII_{(4-11)}$, we currently have limited information regarding the role played by this residue in URP biological activity. UII and URP share common biological actions, however a body of evidence has shown that the two peptides also exert distinct functions, suggesting that these two endogenous UT ligands might be functionally selective [20–24]. As recently demonstrated by virtual docking, the acidic Asp^4 residue of UII or $UII_{(4-11)}$, and not the corresponding aliphatic Ala^1 of URP, can establish a salt bridge with Arg^{193} of the receptor, creating a different binding environment for the two peptides [25]. Supporting this observation, introduction of a Tyr(mI) moiety in $UII_{(4-11)}$ and URP, which only differ by their N-terminal amino acid (Asp versus Ala, respectively), gave rise to two distinct pharmacological profiles with $[Tyr(mI)^6]$URP acting as a weak UT agonist while $[Tyr(mI)^9]UII_{(4-11)}$ is known as a highly potent UT ligand [13,15]. Such results imply that the Tyr environment in UT may be different for UII and URP following receptor binding. In this context, exploiting this apparent divergence between UII and URP could represent a good opportunity to understand the subtle and pluridimensional pharmacology of UT.

In order to generate insight into the mechanisms of UT recognition and activation by URP, we initiated a structure-activity relationship study at the Tyr^6 position of URP by introducing highly hindered and/or conformationally constrained amino-acids (Fig. 1). Compounds were pharmacologically evaluated for their ability 1) to bind UT, 2) to induce/modulate rat aortic ring contraction and 3) to promote activation of Gq and G_{12} using bioluminescence resonance energy transfer (BRET)-based biosensors. The identification of a biased agonist, *i.e.* $[Pep^6]$URP, reveals a crucial role of this residue in the fine tuning of UT signaling. Furthermore, the ability of this compound to selectively reduce the maximal contractile response of URP but not UII indicates that substitution at position 6 could open up new vistas for development of allosteric modulators specifically targeting URP functions.

2. Material and methods

2.1. Materials

The fluorenylmethyloxycarbamate- (Fmoc-) protected amino-acids, Fmoc-Val-Wang resin and (Benzotriazol-1-yloxy)tris(dimethylamino)phosphonium hexafluorophosphate (BOP) were purchased from Chem-Impex (Wood Dale, IL). Trifluoroacetic acid (TFA), methanol (MeOH), acetonitrile (ACN), diethyl ether, N,N-dimethylformamide (DMF), piperidine and dichloromethane (DCM) were obtained from Fisher Scientific (Nepean, ON). $Na^{125}I$ was purchased from Perkin-Elmer (Montreal, QC). All other chemicals were from Sigma-Aldrich (Mississauga, ON).

2.2. Peptide synthesis

Peptides were prepared by solid-phase peptide synthesis using a Fmoc protection strategy and a commercially available Fmoc-Val-Wang resin (0.62 meq/g). All reactions were conducted under a nitrogen atmosphere. Fmoc deprotection was achieved with 20% piperidine in DMF for 15 min. Washes were performed with DMF (2x), MeOH (2x) and DCM (2x). Coupling of the Fmoc-protected amino-acids (3-equivalent excess based on the original resin substitution) were mediated by BOP (3 eq) and N,N-diisopropylethylamine (DIEA; 6 eq) in DMF for approximately 1 h. Completion of the coupling and deprotection steps was monitored with the qualitative Kaiser test. The phenylacetylenyl side chain was introduced on Boc-[L/D-$Phe(pI)^6$]URP peptidyl-resins using the Sonogashira cross-coupling reaction and a previously

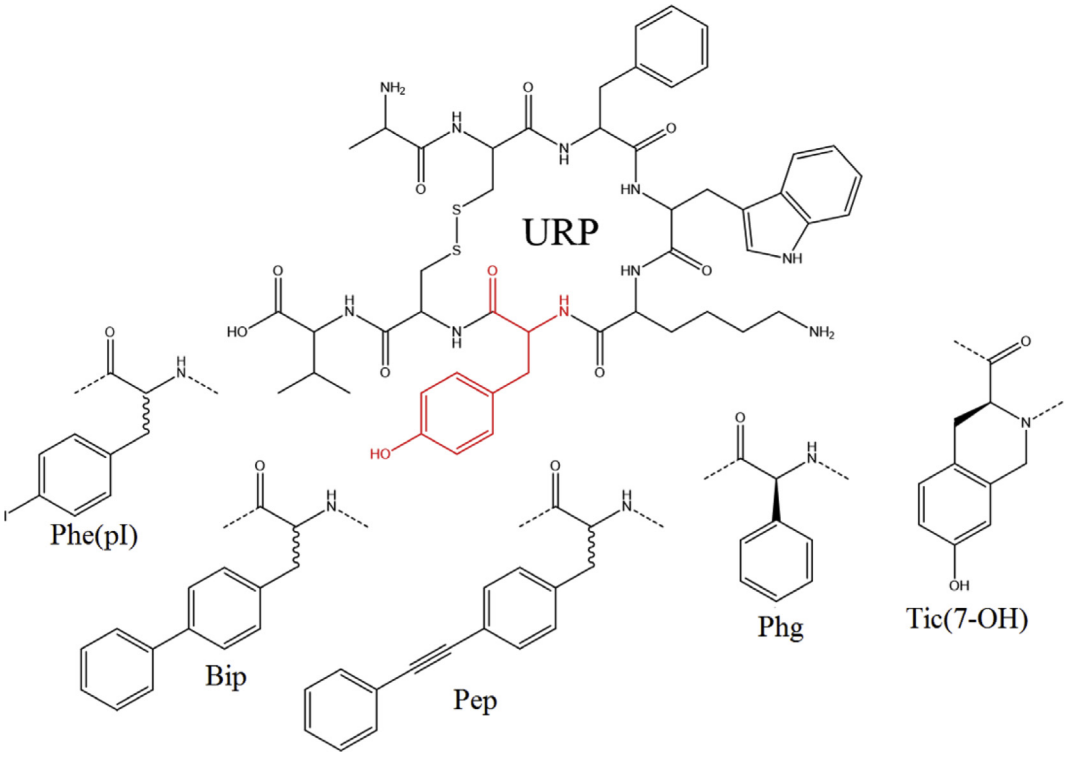

Fig. 1. Structures of the non-natural amino-acids introduced at position 6 in URP. Phe(pI), para-iodo-phenylalanine; Bip, biphenylalanine; Pep, (phenylethynyl)-phenylalanine; Phg, phenylglycine; Tic(7-OH), 7-hydroxy-1,2,3,4-tetrahydroisoquinoline.