Heteromerisation with the bradykinin type 2 receptor enables β-arrestin recruitment to the angiotensin II type 2 receptor

Elizabeth Johnstone[1], Kevin Pfleger[1,2], Mohammed Ayoub[1]. *[1]Western Australian Institute for Medical Research, B Block, QEII Medical Centre, Hospital Avenue, Nedlands, Western Australia, 6009, Australia, [2]Dimerix Bioscience Pty Ltd, B Block, QEII Medical Centre, Hospital Avenue, Nedlands, Western Australia, 6009, Australia*

Bradykinin and angiotensin II are peptide hormones involved in the maintenance of blood pressure. Bradykinin reduces blood pressure via the bradykinin type 2 receptor (B_2R), while angiotensin II increases blood pressure primarily through the angiotensin II type 1 receptor (AT_1R). A second angiotensin II receptor (AT_2R) often, but not always, acts in opposition to the AT_1R, and its molecular and physiological functions remain poorly characterised [1,2]. Previous studies have demonstrated functional crosstalk between AT_2R and B_2R, with a suggested mechanism being heteromerisation [2].

Our aim was to provide evidence for the existence of the AT_2R-B_2R heteromer and investigate its pharmacology. HEK293FT cells were transfected with receptor cDNA fused to Rluc8 (variant of *Renilla* luciferase), untagged receptor, and β-arrestin2 fused to Venus (variant of green fluorescent protein). To investigate the existence of the AT_2R-B_2R heteromer, the G protein-coupled receptor-heteromer identification technology (GPCR-HIT) was employed on the bioluminescence resonance energy transfer (BRET) platform [3,4]. GPCR-HIT utilises ligand-dependent recruitment of interacting proteins such as β-arrestin2 to specifically detect receptor heteromers.

cDNA constructs were generated as described previously for other constructs [5]. AT_2R and V_2R were kindly provided by Walter Thomas (University of Queensland, Australia) and Thierry Durroux (Institute of Functional Genomics, Montpellier, France), respectively. B_2R was obtained from the Missouri S&T Resource Center. Transient transfections were carried out using GeneJuice (Merck, Kilsyth, Australia) and all BRET measurements were taken at 37°C.

Bradykinin treatment of cells co-expressing AT_2R-Rluc8, β-arrestin2-Venus and B_2R resulted in a ligand-dependent BRET signal (0.172 ± 0.011, n=3) indicative of recruitment of β-arrestin2 to the heteromer. No signal was obtained when only AT_2R-Rluc8 and β-arrestin2-Venus were expressed, following treatment with either bradykinin (0.003 ± 0.007, n=3) or angiotensin (0.0005 ± 0.006, n=3). The GPCR-HIT signal was specific for the AT_2R-B_2R heteromer as the signal obtained when AT_2R-Rluc8 and β-arrestin2-Venus were co-expressed with the vasopressin type 2 receptor was significantly smaller (0.043 ± 0.020, n=3, $p < 0.001$, two-way ANOVA compared to signal with AT_2R-Rluc8, β-arrestin2-Venus and B_2R). Subsequent antagonist assays showed that the bradykinin-induced BRET signal (0.153 ± 0.028, n=3) could be significantly inhibited with a 30 minute pretreatment of the B_2R antagonist HOE140 (0.017 ± 0.025, n=3, $p < 0.05$, one-way ANOVA) indicating that B_2R activation was required for recruitment of β-arrestin2 to the AT_2R-B_2R heteromer.

This study provides evidence for the existence of the AT_2R-B_2R heteromer. β-arrestin2 recruitment proximal to the AT_2R requires the specific co-expression and activation of the B_2R.

1. Porrello ER, Pfleger KD, Seeber RM, Qian H, Oro C, Abogadie F, Delbridge LM, Thomas WG (2011) Heteromerization of angiotensin receptors changes trafficking and arrestin recruitment profiles. *Cell. Signal.* 23: 1767-1776

2. Porrello E, Delbridge L, Thomas W (2009) The angiotensin II type 2 (AT2) receptor: an enigmatic seven transmembrane receptor. *Front. Biosci.* 14: 958-972

3. See HB, Seeber RM, Kocan M, Eidne KA, Pfleger KD (2011) Application of G Protein-Coupled Receptor Heteromer Identification Technology to monitor β-arrestin recruitment to G protein-coupled receptor heteromers. *Assay Drug Dev. Technol.* 9: 21-30

4. Mustafa S, See HB, Seeber RM, Armstrong SP, White CW, Ventura S, Ayoub MA, Pfleger KD (2012) Identification and profiling of a novel α1A-adrenoceptor-CXC chemokine receptor 2 heteromer. *J. Biol. Chem.* 287: 12952-12965

5. Kocan M, See H, Seeber R, Eidne K, Pfleger KDG (2008). *Journal of Biomolecular Screening* 13: 888-898