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

Elizabeth Johnstone1, Kevin Pfleger1,2, Mohammed Ayoub1. 1Western Australian Institute for Medical Research, B Block, QEII Medical Centre, Hospital Avenue, Nedlands, Western Australia, 6009, Australia, 2Dimerix 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 (B2R), while angiotensin II increases blood pressure primarily through the angiotensin II type 1 receptor (AT1R). A second angiotensin II receptor (AT2R) often, but not always, acts in opposition to the AT1R, and its molecular and physiological functions remain poorly characterised [1,2]. Previous studies have demonstrated functional crosstalk between AT2R and B2R, with a suggested mechanism being heteromerisation [2].

Our aim was to provide evidence for the existence of the AT2R-B2R 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 AT2R-B2R 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]. AT2R and V2R were kindly provided by Walter Thomas (University of Queensland, Australia) and Thierry Durroux (Institute of Functional Genomics, Montpellier, France), respectively. B2R 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 AT2R-Rluc8, β-arrestin2-Venus and B2R 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 AT2R-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 AT2R-B2R heteromer as the signal obtained when AT2R-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 AT2R-Rluc8, β-arrestin2-Venus and B2R). 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 B2R antagonist HOE140 (0.017 ± 0.025, n=3, p < 0.05, one-way ANOVA) indicating that B2R activation was required for recruitment of β-arrestin2 to the AT2R-B2R heteromer.

This study provides evidence for the existence of the AT2R-B2R heteromer. β-arrestin2 recruitment proximal to the AT2R requires the specific co-expression and activation of the B2R.

