Proceedings of the British Pharmacological Society at http://www.pA2online.org/abstracts/Vol18Issue1abst212P.pdf

Defining the biology of FPR2 receptor; pro-resolving signalling and function

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Introduction: The human formyl peptide receptor (FPR) family comprises of 3 members. FPR2 is a unique receptor, activated by both pro-resolving and pro-inflammatory agonists and is one of the most multifaceted GPCRs characterised to date [1]. For the first time we have provided an explanation for the molecular mechanisms of FPR2 [2]. We revealed a constitutive, agonist independent, dimerisation of FPR2. A conformational change of the dimer was provoked by the pro-resolving agonists AnxA1 and Lipoxin A4, but not by the pro-inflammatory agonists LL-37 or SAA. We identified a cascade downstream of dimer activation mediated by p38, MAPKAPK and Hsp-27. These data suggest biased agonism at the receptor; a conformational change within the FPR2 homodimer elicited by AnxA1 is responsible for the functional selectivity leading to pro-resolving responses. Sequence-structural analyses of FPR2 revealed the presence of features, which are conserved across GPCRs, including at least two putative dimerisation sites: 1) a chemokine receptor dimerisation motif GxxxLxxL (residues G21-L25-L28) located in FPR2 N-terminus, and 2) GxxGxxL within TM1 (G40-G43-L46).

Method: To determine how disruption of these motifs impact its regulation we used site-directed mutagenesis. We generated a triple mutant replacing all 3 residues with Ala ($FPR2_{A21A25A28}$ and $FPR2_{A40A43A46}$) using FPR2x3HA and FPR2x3Flag constructs previously created in the lab. Cell surface trafficking was determined using confocal microscopy and fluorescence associated cell sorting (FACS). Receptor dimerisation and signalling were studied using Co-immunoprecipitation (co-IP) and Western blotting. Experiments were repeated 3 times with distinct cell preparations.

Results: Using confocal microscopy and FACS on transfected HEK293 cells, we show no difference in cell surface expression between FPR2 WT and $FPR2_{A21A25A28}$ mutants. Mutation of all three residues impaired receptor dimerisation as determined by co-IP. Altered Hsp27 signalling was observed in cells expressing the triple mutant compared to WT when stimulated with AnxA (100nM). Preliminary data targeting the second putative dimerisation motif, G40-G43-L46, show significant decrease in cell surface expression of cells transfected with the triple mutant ($FPR2_{A40A43A46}$) compared to WT.

Conclusions: The two putative motifs appear to have different impacts on FPR2 function. Disruption of G21-L25-L28 motif does not affect cell surface trafficking but affects function whilst disruption of the G40-G43-L46 motif affects FPR2 cell surface expression. Further work will help establish the role of these dimerisation motifs on FPR2 function.

References

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