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Systematic mutation reveals different phospho-interaction patterns of beta-arrestin-1 to GPR35 and GPR120

L. Lin, A. Tobin, G. Milligan. Institute of Molecular, Cell and Systems Biology, Glasgow, United Kingdom.

Introduction: Decreased G protein-coupled receptor (GPCR) internalization or highly-activated β-arrestin-biased signalling is closely associated to diseases such as cardiovascular disease or cancer respectively [1,2]. Further investigation of GPCR-β-arrestin interaction may therefore be important for understanding the mechanism(s) involving in the development and progression of these diseases. Currently, the receptor phospho-barcode and how this modulates the interaction pattern with β-arrestins are poorly understood. In a previous report, seven phosphate-binding sites of β-arrestin-1 were identified by an NMR approach [3]. Here, we have generated seven β-arrestin-1 mutants corresponding to these sites and demonstrate: 1) GPR35 and GPR120 interact with different phosphate-binding sites of β-arrestin-1; 2) some mutations of β-arrestin-1 lead to greater levels of GPCR-β-arrestin-1 interaction.

Methods: GPR35 and GPR120 receptor constructs contained enhanced yellow fluorescent protein (eYFP) fused to the C terminus as previously described [4,5]. Constructs of wild type and mutant bovine β-arrestin-1 with NanoLuc luciferase fused to their N terminus were generated and applied in bioluminescence resonance energy transfer (BRET) assays. β-arrestin-1 mutations include site1 (Y63A/R65A/K77A), site2 (K160A/R65A/R165A), site3 (K160A/K11A), site4 (R25A), site5 (R7A), site6 (R7A), and site6/7 (Y21A/K107A/K10E). Agonists applied here were zaprinast for GPR35 and TUG-891 for GPR120. Results represent the mean±SEM.

Results: Results are shown as Table 1:
1) GPR35 interacts with sites 1, 2, 3, 4 of β-arrestin-1, whereas GPR120 interacts with sites 2, 3, 6.
2) Site 7 displays species selective β-arrestin-1 interactions between mouse and human GPR35
3) Site 6 or sites 1, 5 significantly increase β-arrestin-1 interactions with GPR35 or GPR120 respectively.

Table 1. β-arrestin-1 recruitment (% max WT response).

	WT	**SITE1**	**SITE2**	**SITE3**	**SITE4**	**SITE5**	**SITE6**	**SITE6/7**
h35	100.0±5.0	33.8±3.6	46.0±5.0	15.8±4.0	59.7±2.7	100.7±1.5	140.0±1.4	196.4±7.7
m35	100.0±3.1	53.8±7.8	51.3±4.2	17.7±2.5	29.0±2.8	109.7±4.0	188.5±3.4	69.9±3.0
h120	100.0±3.4	177.8±0.3	66.3±0.3	61.4±2.8	126.7±6.4	200.1±4.7	73.9±1.9	106.6±7.5
m120	100.0±0.2	158.9±1.4	52.1±2.9	47.7±2.3	113.7±0.9	137.4±0.8	78.8±2.6	123.3±5.2

Conclusions: β-arrestin-1 displays different phospho-interaction patterns with GPR35 and GPR120. These differences may allow β-arrestin-1 to activate different downstream targets and contribute to the functional diversity of GPCR receptors in their control of physiological responses.

References:
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