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An extracellular loop 2 mutation P198A prevents the action of the antagonist RC3095 at the gastrin releasing peptide BB2 receptor

L. F. Medeiros[1], D. Nesheva[1], H. Verli[2], R. Roesler[3], N. D. Holliday[1]. [1]School of Life Sciences, University of Nottingham, Nottingham, UNITED KINGDOM, [2]Centro de Biotecnologia, Universidade Federal do Rio Grande do Sul, Porto Alegre, BRAZIL, [3]Department of Pharmacology, Universidade Federal do Rio Grande do Sul, Porto Alegre, BRAZIL.

Introduction. Antagonists for Gastrin releasing peptide (GRP) receptor BB2 have therapeutic potential, for example in analgesia and prevention of tumour proliferation (1,2). Previously, the extracellular loop 2 mutation P198A has been reported to decrease binding affinity for the BB2 agonist peptide bombesin (3). Here we explore the role of Pro198 in the action of GRP, bombesin and the antagonist RC3095 ([D-Tpi^{6},Leu^{13}y(CH2-NH)-Leu^{14}]bombesin$^{6-14}$]) (4) at the BB2 receptor.

Methods. HEK293 cells stably expressed SNAP-tagged human BB2 receptor (HEK BB2) or P198A mutant and fluo4 calcium mobilisation measurements were performed as previously described (5). Briefly cells on 96 well imaging plates were loaded using DMEM containing 1.5µM Fluo-4 and 2.5mM probenecid. Antagonist incubations (5 min, 37℃) and agonist treatments were in HEPES buffered saline solution / 0.1% BSA / probenecid, with fluo4 fluorescence was measured on an MDC Flexstation. Concentration response curves were constructed from pooled data using peak agonist responses (Graphpad Prism v7). Pooled pEC_{50} and R_{max} values are mean±s.e.m. from 5-7 experiments, with differences between data groups tested by one way ANOVA followed by Dunnett's post test.

Results. GRP and bombesin stimulated calcium mobilisation in HEK BB2 cells with pEC_{50} values of 9.59±0.19 and 10.30±0.22 respectively, and similar R_{max} (2.86±0.19 fold over basal for GRP and 2.96±0.15 for bombesin). There were small decreases ($P<0.05$) in agonist potency for HEK BB2 P198A calcium responses (GRP pEC_{50} 8.92±0.18, bombesin pEC_{50} 9.63±0.18), with R_{max} values unaffected. RC3095 was a non-surmountable antagonist of GRP calcium responses in HEK BB2 cells, but in contrast this antagonist was without significant effect on GRP responses in HEK BB2 P198A cells (Figure 1).

Conclusions. These data confirm the modest effect of P198A on agonist activation of the BB2 receptor (3), and for the first time identify its critical role in the action of RC3095 as a BB2 receptor antagonist. These inhibitory effects might be attributable to a direct influence on the ligand binding site, or more global effects on BB2 extracellular loop 2 structure.

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References. 1. Roesler R and Schwartzmann G (2012). *Front Endocrinol* **3**: 159. 2. Jensen RT *et al*. (2008). *Pharmacol Rev* **60**: 1-42. 3. Akeson M *et al*. (1997). *J Biol Chem* ***272***: 17405-17409. 4. Radulovic S *et al*. (1991). *Int J Pept Protein Res* **38**: 593-600. 5. Watson SJ *et al*. (2012). *Mol Pharmacol* **81**: 631-642.

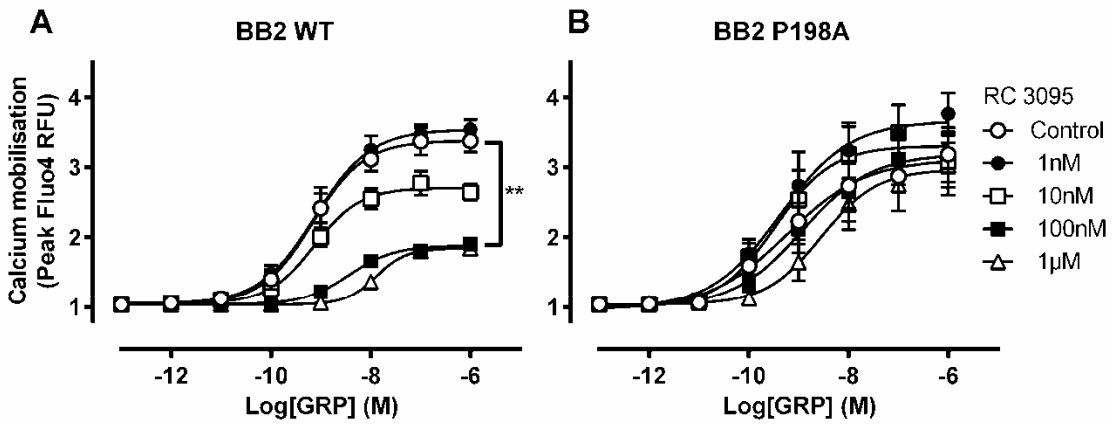

Figure 1: GRP concentration response curves in the calcium mobilisation assay for HEK BB2 (A) and HEK BB2 P198A cells (B), +/- increasing concentrations of RC 3095. Data represent mean ± SEM of 5-7 experiments; **P<0.01 for R_{max} relative to control in the absence of antagonist (one-way ANOVA/ Dunnett's test).