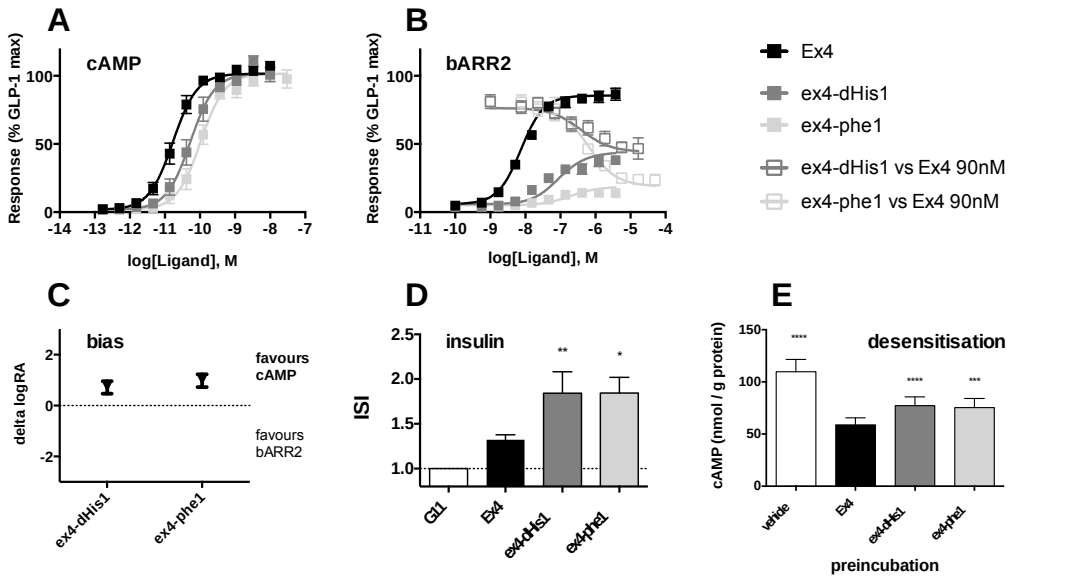
Improving GLP-1 receptor biological activity in pancreatic beta cells via biased agonism

Exendin-4 (Ex4) is a glucagon-like peptide-1 (GLP-1) mimetic used in the treatment of type 2 diabetes. Binding of Ex4 to the GLP-1R results in recruitment of both G proteins and β-arrestins, which have been implicated in signalling to divergent downstream biological events including potentiation of insulin secretion and reduction in β-cell apoptosis. We therefore sought to develop peptide analogues of Ex4 with bias between G protein and arrestin signalling, which might possess therapeutically useful characteristics. Analogue-induced β-arrestin-2 (βARR2) recruitment and cyclic AMP (cAMP) generation were tested in Pathhunter CHO-GLP-1R cells and these data used to calculate pathway-specific relative activities for each agonist (1). N-terminal substitutions to Ex4 led to 8-10-fold bias towards cAMP and away from βARR2 recruitment. Despite also exhibiting reduced potency for cAMP, test analogues were approximately twice as insulinotropic as Ex4 when administered to β-cells over 24 hours. This was associated with a 30% reduction in GLP-1R desensitisation in comparison to Ex4. These findings are in keeping with the canonical role of arrestins in mediating receptor desensitisation, and may have implications for development of GLP-1R mimetics with improved long-term efficacy.



A: cAMP response after 30 minute stimulation in CHO-GLP-1R cells, n=6, with Ex4 +/- N-terminal substitution (L-histidine to D-histidine [ex4-dHis1] or phenylalanine [ex4-phe1]. **B**: bARR2 recruitment after 90 minute stimulation in CHO-GLP-1R cells, n=6. **C**: Pathway bias (log scale) relative to Ex4 using data from A and B. 95% CI indicated. **D**: Insulin secretion in INS-1 832/3 cells after 24hr incubation at 11mM glucose (G11), n=6. Peptides administered at 100nM and response expressed as insulin stimulation index (ISI) relative to G11. *P<0.05, ** p<0.01 vs Ex4 using randomised block ANOVA + Friedman test. **E**: cAMP response to 10nM GLP-1 in INS-1 832/3 cells after prior exposure to 100nM Ex4, test analogue or vehicle for 30 minutes, n=6. ****p<0.0001 vs Ex4 using randomised block ANOVA + Sidak test.

1) Stahl *et al*. (2015). *Mol Pharmacol*. **87**:866–77.