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Investigating the importance of gcgr icl1 region and helix 8 for cell-surface expression and signalling

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Introduction The glucagon receptor (GCGR) regulates blood glucose levels through its ability to bind the peptide hormone glucagon. This leads to the stimulation of glycogenolysis and gluconeogenesis in the liver, effectively counteracting the consequences of excessive insulin (1). In this work, an investigation into the importance of GCGR residues for cell-surface expression and downstream signalling components (cAMP, pERK1/2 and Ca^{2+}i mobilisation) was performed. These included ICL1 region ($G165^{1.63}$-$T172^{2.45}$), TM2 residue $R173^{2.46}$ and helix 8 residues $E406^{8.49}$ and $E410^{8.53}$.

Method GCGR ICL1 mutants were generated using QuikChange Lightening Site-Directed Mutagenesis Kit (Agilent Technologies). Wild-type (WT) or mutant GCGR cell-surface expression was determined in transiently transfected HEK 293 cells through FACS analysis. Downstream signalling following GCG stimulation was quantified using LANCE® cAMP Detection Kit (PerkinElmer), (HTRF)® Phospho-ERK (T202/Y204) Cellular Assay Kit (Cisbio Bioassays) or Fluo-8, AM dye (Stratech Scientific) allowing the measurement of intracellular Ca^{2+} (Ca^{2+}i) mobilisation. Specific residues were mutated within the currently available GCGR structures (PDB ID: 5XF1, 5XEZ, 5EE7 and 4L6R) using Modeller (version 9.18), to aid in interpretation of the experimental results.

Results With the exception of C171A, which showed no apparent cell-surface expression or response, and G165A, which showed a significantly reduced maximal cAMP response (E_{max} 73.4 ±3.7), ICL1 mutants showed negligible differences in cell-surface expression, pERK1/2 or cAMP accumulation when compared to WT GCGR. In contrast, K168A, L169A, H170A and T172A showed a reduced maximum $G_{q/11}$-mediated Ca^{2+}i mobilisation (E_{max} 65.2 ±5.5, 54.4 ±4.8, 50.9 ±4.9, and 70.0 ±5.3 percentage WT). R173A showed similar cell-surface expression but a severely attenuated GCG potency. E406A and E406A E410A showed significantly reduced cell-surface expression (53.8 ±5.8 and 21.5 ±1.3 percentage WT cell-surface expression, respectively) but enhanced constitutive activity (basal 11.1 ±1.5 and 19.6 ±1.4, respectively) when compared to WT (basal 7.6 ±1.1).

Conclusion The findings suggest that K168, L169, H170 and T172 may play a role in Ca^{2+}i mobilisation, possibly through direct interactions with $G_{q/11}$. In contrast, R173 appears to be important for G_s-mediated signal transduction. The lost polar interaction with E406 in modelled R173A GCGR may suggest this interaction is critical for GCGR stability. Indeed, the enhanced constitutive activity in helix 8 GCGR mutants may be explained by a reduced inactive state stability or an enhanced ability of R173, now released from E406A, to interact with the G protein more freely in the absence of ligand.

References

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