Proceedings of the British Pharmacological Society at http://www.pA2online.org/abstracts/Vol19Issue1abst025P.pdf

Homo- and heterooligomerization of the incretin receptors

S. Al-Sabah[1], M. Bünemann[2], C. Krasel[2]. [1]Dept. of Pharmacology and Toxicology, Faculty of Medicine, Kuwait University, Kuwait, Kuwait, [2]Institute for Pharmacology and Clinical Pharmacy, Philipps-University Marburg, Marburg, Germany.

Introduction The incretins glucagon-like peptide 1 (GLP1) and gastric inhibitory peptide (GIP) are secreted by cells in the gut in response to food ingestion. Both peptides promote the secretion of insulin from beta cells of the pancreas through specific G-protein-coupled receptors (the GLP1 and GIP receptor, respectively). As both receptors occur on the same cell, there is at least the hypothetical possibility that these receptors might interact.

Method We used Fluorescence Resonance Energy Transfer (FRET) and two-colour Fluorescence Recovery After Photobleaching (FRAP) to directly investigate receptor interaction. We also used FRET to measure receptor interaction with arrestins. All experiments were performed in transiently transfected HEK293T cells.

Results In HEK293T cells transiently transfected with YFP- and CFP-tagged GLP1 receptor, the application of 1 µM GLP1 caused a reduction in FRET between the two fluorophores. In contrast, 1 µM GLP1 caused an increase in FRET between CFP-tagged GLP1 receptor and YFP-tagged GIP receptor whereas 1 µM GIP caused a decrease in FRET between the two receptors. For two-colour FRAP, HEK293T cells were transiently transfected with YFP- and CFP-tagged GLP1 receptor. The YFP-tagged receptor also carried an extracellular Flag tag. Cells were treated with a polyclonal anti-Flag antibody followed by crosslinking with a secondary antibody to immobilize the YFP-tagged receptors. This crosslinking also reduced the lateral mobility of the CFP-tagged receptors, again suggesting that the CFP- and YFP-tagged receptors interact. Finally, to investigate whether oligomerization might alter receptor signaling, we investigated arrestin recruitment by the incretin receptors. In our hands, agonist stimulation of the GLP1 receptor robustly induces arrestin recruitment in a FRET assay whereas the GIP receptor does not recruit arrestin (1). However, when cells were transfected with YFP-tagged GIP receptor, untagged GLP1 receptor and CFP-tagged arrestin3, 1 µM GIP treatment now was able to induce recruitment of arrestin3 to the GIP receptor as measured by FRET.

Conclusions Taken together, our data suggest that GLP1 receptors may form homo-oligomers or hetero-oligomers with GIP receptors. Application of agonist could trigger size changes in the oligomer but more likely changes the relative orientation of the individual receptors in a given oligomers. This oligomerization might be relevant for incretin signal transduction on pancreatic beta cells.

References

(1) S. Al-Sabah et al. (2014) *PLoS One* **9**: e106890.