ESTABLISHING SIGNALING ASSAYS TO DETERMINE THE BIASED AGONISM OF THE RELAXIN FAMILY PEPTIDE RECEPTOR 3

S. Senanayake[1], D. Bethmage[1] and T. Jayakody[1*]
[1]Department of Chemistry, Faculty of Science, University of Colombo, Sri Lanka

G-protein-coupled receptors (GPCRs), which make up the largest protein superfamily in the human body, are essential for cellular signaling. They are essential pharmacological targets for the development of drugs. The relaxin family peptide receptor 3, commonly known as relaxin-3 receptor 1 (RXFP3), belongs to the class A GPCR group. It is prominently expressed in brain regions such as the hypothalamus, amygdala, and the brainstem. The signaling pathways activated by the RXFP3 receptor upon binding with its ligands are inhibition of cyclic adenosine monophosphate (cAMP) accumulation, ERK1/2, MAPK, and beta-arrestin recruitment. However, additional information is needed to determine the distinct preferences of different RXFP3 ligands regarding their recruitment of beta-arrestin or activation of G-proteins. One of the upstream effectors activated by the RXFP3 receptor is $G\alpha_{i/o}$, which is the primary focus of this work. The objective is to establish a GloSensor™ cAMP signaling assay to determine the functional selectivity of RXFP3 receptor activation by relaxin-3 agonists and its inhibition of forskolin-induced cAMP assays. In this method, a luciferase enzyme connected to a cAMP-responsive component is used. The luciferase enzyme is triggered when cAMP levels rise, producing light. This luminescence provides a reliable measure of $G\alpha_{i/o}$ activation via RXFP3.In comparison to existing cAMP measurement assays like Enzyme-Linked Immunosorbent Assay (ELISA), the GloSensor™ cAMP assay, being a luminescence-based technique has several benefits, such as being cost-effective and efficient. In the initial stages of the study, we isolated and validated a pCAGGS mammalian expression plasmid construct containing the GloSensor coding sequence. Then, the study focused on HEK-RXFP3 cell line-based forskolin-induced cAMP assays. Adenylate cyclase activation and cAMP signaling in cells were investigated using the well-known stimulant forskolin. The assay detected the highest luminescence signal in response to forskolin and 3-Isobutyl-1-methylxanthine (IBMX), after transfecting HEK-RXFP3 cells with the GloSensor plasmid, whereas the lowest signal was seen at the highest relaxin-3 concentration. The resulting dose-response curve showed fluctuation of cAMP that was released in response to relaxin-3 ligands. It is interesting to note that lower relaxin-3 concentrations showed the greatest cAMP accumulation. During the optimization phase, experimental parameters, including PEI concentration (1mg/ml) and cell count per well, were fine-tuned. The results revealed that 5 µl (25 µg/ml) of branched PEI yielded the most robust signal compared to other concentrations. Additionally, relaxin-3 demonstrated the ability to reduce cAMP synthesis by activating Gαi/o inhibitory pathways through RXFP3 binding, highlighting the receptor's modulation of both stimulatory and inhibitory pathways.

Keywords: *GPCR, RXFP3, Relaxin 3, pCAGGS, GloSensor™ cAMP assay.*