Characterisation of Inositol Phosphate & cAMP Signalling from GPCRs using Homogeneous Time-Resolved Fluorescence Technology

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Introduction

This study has utilised CisBio's HTRF IP-One and cAMP Assay Kits to investigate the Gq and Gs signalling properties of various G protein-coupled receptors (GPCRs).

The high quality of data has enabled phenomena such as partial agonism and biased signalling to be identified.

Bradykinin type 2 receptor (B_2R) Gq signalling.

The B_2R regulates blood pressure through bradykinin (BK)-induced vasodilation. It preferentially couples to Gq proteins resulting in the production of IP.

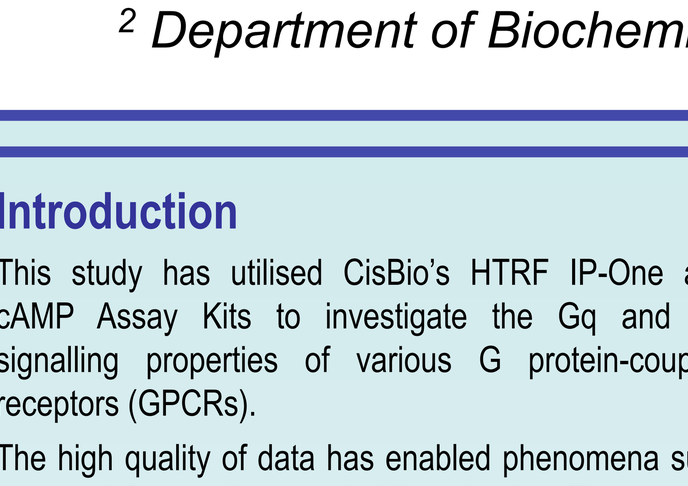

Figure 1: Partial agonism of the B_2R 'antagonist' HOE140.

HEK293FT cells were transfected with HA-B_2R and treated with 0.1 µM BK, with or without pretreatment with the B_2R 'antagonist' HOE140. Despite its classification as an antagonist, at moderate doses (EC_{50} = 42 nM) HOE140 acted as an agonist (red curve), inducing the production of IP through B_2R. However, at higher doses it was able to inhibit 0.1 µM BK-induced IP production (green curve). **These results demonstrate the partial agonism of HOE140.**

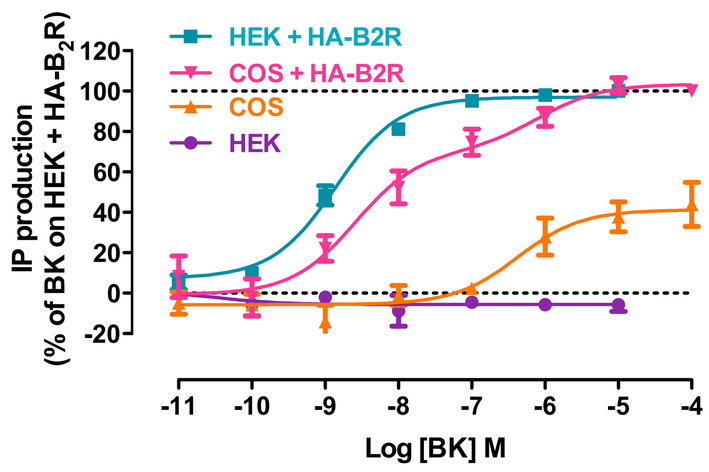

Figure 2: Endogenous expression of BK receptors in COS7 but not HEK293FT cells.

HEK293FT and COS7 cells were transfected or not with HA-B_2R and treated with BK to determine the presence of endogenous BK receptors. HEK293FT cells transfected with B_2R show a dose dependent production of IP that is not observed in untransfected cells. COS7 cells transfected with B_2R also show a dose dependent production of IP, however, the fit of a two-site curve suggests the presence of two different affinity BK sites. In contrast to HEK293FT cells, untransfected COS7 cells produce IP in a BK-dose dependent manner, indicating the presence of endogenous BK receptors. **These results indicate the presence of endogenous BK receptors in COS7 cells.**

Gs and Gq signalling properties of vasopressin type 2 receptor (V_2R) mutants.

The V_2R controls water and salt homeostasis through the antidiuretic actions of its effector, vasopressin (AVP). It preferentially couples to Gs proteins resulting in production of cAMP, and to a lesser extent it is also able to couple to Gq proteins. Here, three mutations identified in patients have been investigated: R181C and M311V that are associated with loss of function and V266A investigated for potential association with a gain-of-function phenotype.

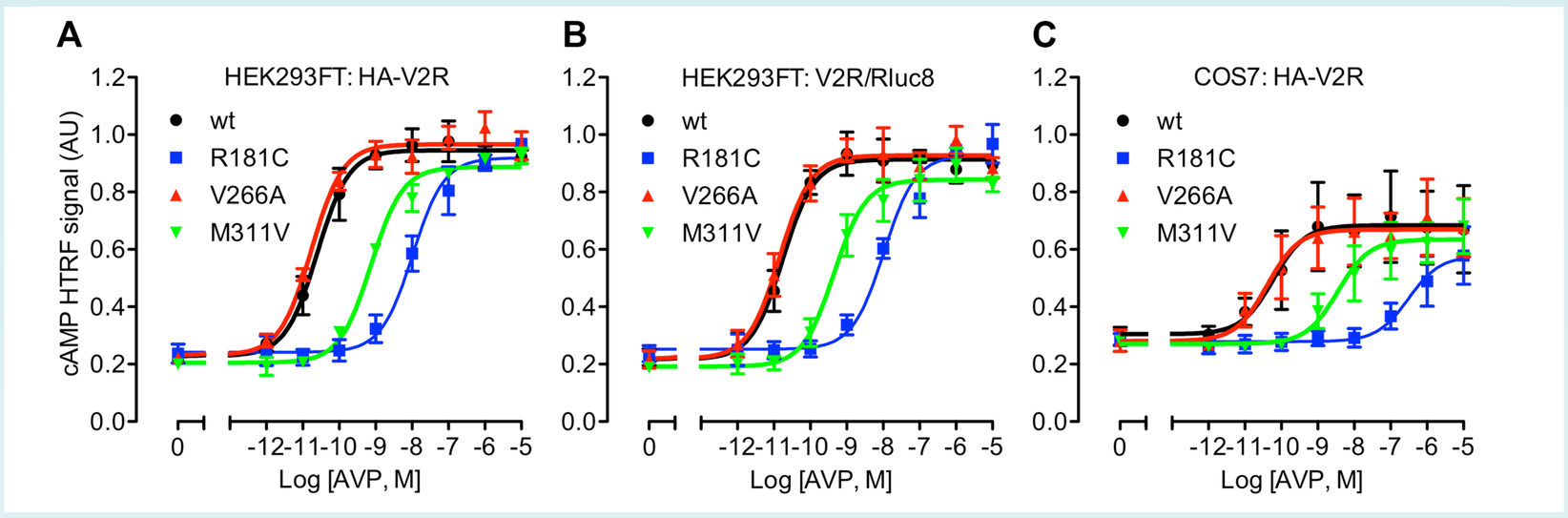

Figure 3: AVP-induced cAMP production with V_2R mutants.

HEK293FT (A and B) or COS7 (C) cells were transfected with wild type or mutant HA-V_2R (A and C) or V_2R-Rluc8 (B) and treated with AVP. A dose-dependent production of cAMP was observed with the wild type receptor, which was not significantly different from that observed with the V266A mutant (A). In contrast the R181C and M331V mutants produced a significantly lower potency of response. The same trend was observed with V_2R-Rluc8 receptors (B). Despite a lower efficacy of cAMP production in COS7 cells (C) when compared with HEK293FT cells, a similar trend was again observed, this time with even greater decreases in potency. **These results illustrate that while the V266A mutant does not have an altered production of cAMP, the R181C and the M311V mutants display a much weaker response to AVP. Importantly, the M311V mutant retains greater activity than the R181C mutant, which is consistent with the milder disease phenotype associated with this mutant.**

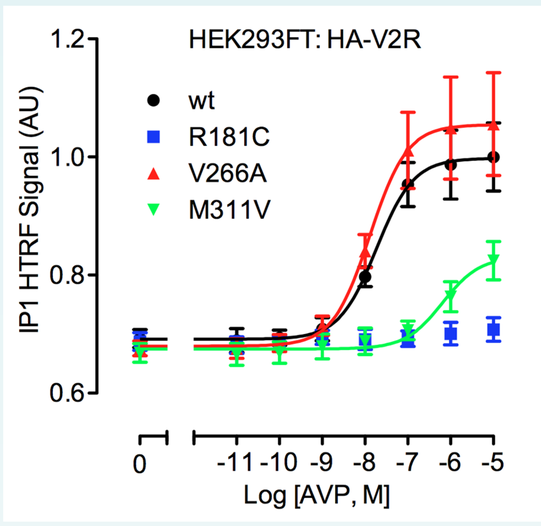

Figure 4: AVP-induced IP production with V_2R mutants.

HEK293FT cells were transfected with wild type or mutant HA-V_2R and treated with AVP. While a dose-dependent production of IP was observed with the wild type receptor, the potency of this response was shifted to the right of the cAMP response (Fig. 3). Compared to the wild type, the V266A mutant did not produce a significantly different IP response whereas the response of the M311V mutant was markedly reduced. AVP was unable to induce IP production in cells expressing the R181C mutant at any of the doses tested. **These results demonstrate the partial agonism of AVP in terms of IP production at the M311V mutant. Additionally, the R181C mutant appears to be a Gs biased receptor that is unable to couple and signal to Gq.**

Angiotensin II type 1 receptor (AT_1R) and chemokine CC receptor 2 (CCR2).

AT_1R regulates blood pressure through angiotensin II (AngII)-induced vasoconstriction. It preferentially couples to Gq proteins resulting in the production of IP. CCR2 primarily mediates monocyte chemotaxis through its effector monocyte chemoattractant protein-1 (MCP-1). It preferentially couples to Gi, but in addition to inhibiting cAMP production, Gi activation can result in IP production through activation of phospholipase Cβ via the βγ subunits.

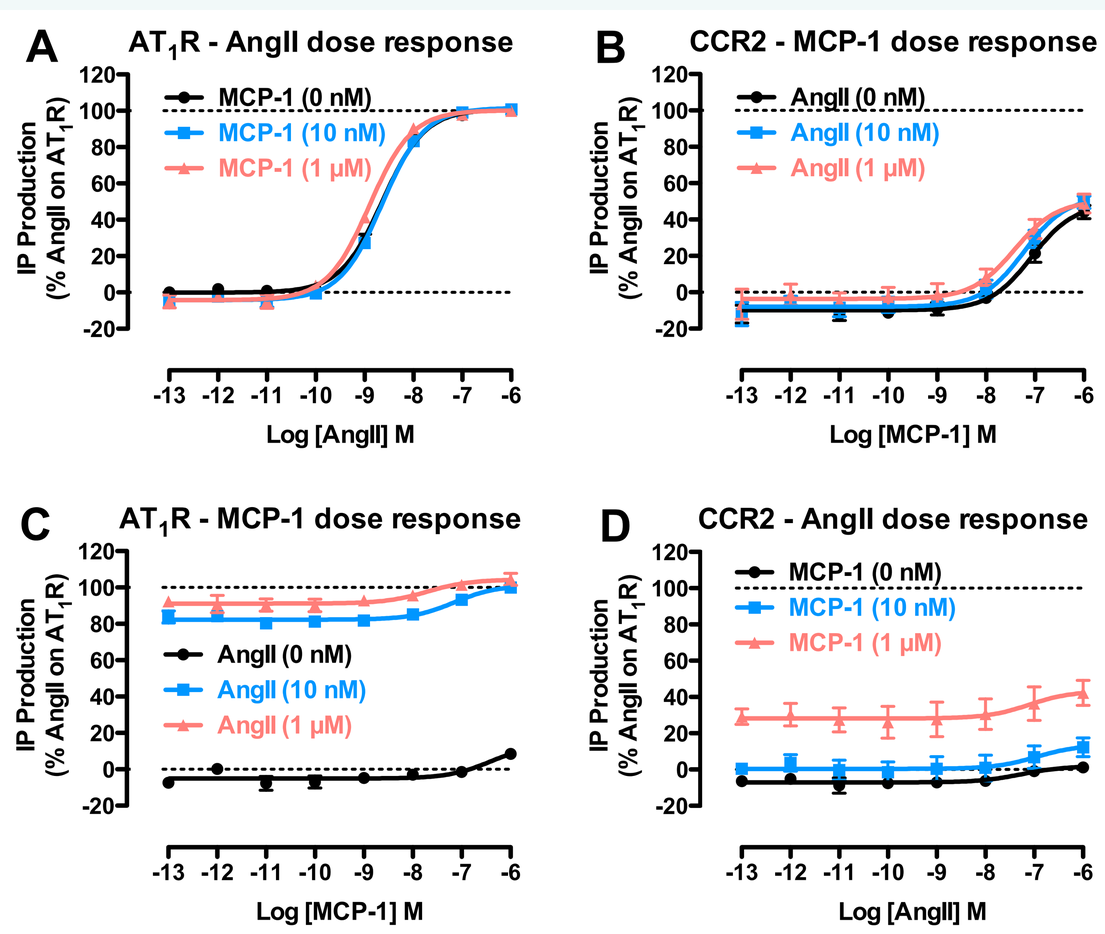

Figure 5: AngII- and MCP-1-induced IP production.

HEK293FT cells were transfected with AT_1R (A and C) or CCR2 (B and D) and treated with AngII and MCP-1. The dose-dependent production of IP with AT_1R (A) and CCR2 (B) when treated with their cognate agonists is not altered through combined treatment with the second ligand. As expected for a preferentially Gi coupled receptor, the potency of IP production for CCR2 is lower than for AT_1R. Similarly in the reverse receptor-treatment configuration (AT_1R (C) and CCR2 (D)) there is negligible IP production as a result of treatment with the other receptor's agonist, while the two doses of cognate ligand produce comparable responses to what was observed in graphs A and B.