Utilization of PathHunter™ Arrestin Cell Lines for Detection of Arrestin Biased Ligands and Compound Pharmacology Distinct from Second Messengers

Tom Wehrman,Qumber Jafri, Daniel Bassoni and Keith R. Olson
DiscoveRx Corporation, Fremont, CA 94538, USA.

Abstract

DiscoveRx has developed a panel of over 120 characterized GPCR cell lines that monitor receptor activity using interaction with human β-Arrestin. These cell lines have numerous applications in both high throughput screening and downstream compound analysis, and have now been successfully adopted by a number of pharmaceutical and biotechnology companies. A relatively recent application for these cell lines has been analysis of compounds for arrestin ligand bias. We have demonstrated that the cells can be used to detect arrestin biased compounds for two example GPCRs, providing additional value for this product panel. As these cells have been used more frequently in profiling applications, we have identified a number of examples where the compound pharmacology is distinct with an arrestin readout compared to more traditional second messenger assays. We will present specific examples where compounds have shown unique pharmacology when analyzed with arrestin. Taken together, the results here suggest that arrestin is a valuable, complementary technology for analysis of GPCR function and indicate that some receptors may merit analysis with both arrestin and more traditional second messenger approaches to obtain a complete understanding of compound function.

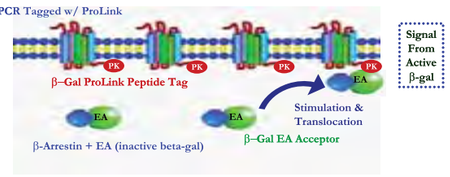

Figure 1. Arrestin-GPCR Assay Principle

- A homogeneous complementation assay for protein-protein interaction
- Monitors arrestin binding to activated GPCRs
- Requires introduction of a ProLink tagged GPCR into a clonal cell line expressing arrestin fused to the EA acceptor fragment of β-Gal

45 GPCR Families Available ($G_{i,q,s}$ - linked, Type A & Type B)

5-Hydroxytryptamine (2)	Calcitonin (2)	Ghrelin (1)	Neuromedin U (1)	Prokineticin (2)
Acetylcholine (4)	Cannabinoid (2)	Glucagon (5)	Neuropeptide Y (3)	Protease-activated (4)
Adenosine (3)	Chemokine (17)	Histamine (3)	Nicotinic acid (1)	Prolactin releasing (1)
Adrenoceptor (2)	Cholecystokinin (2)	Leukotriene (1)	Opioid (3)	Somatostatin (4)
Angiotensin (2)	Corticotropin releasing (2)	Lysophosphoβpid (5)	Orexin (2)	Tachykinin (1)
Anaphylotoxin (1)	Dopamine (2)	Melanocortin (4)	Purinergic (3)	Thyrotropin release (1)
Apelin (1)	Endothelin (2)	Melanin concentrating (1)	Parathyroid hormone (2)	Urotensin (1)
Bombesin (1)	Formyl peptide (2)	Motilin (1)	Platelet activating factor (1)	Vasopressin (2)
Bradykinin (1)	Galanin (2)	Neurotensin (1)	Prostanoid (5)	VIP & PACAP (3)

Table 1. PathHunter™ GPCR Arrestin Cell Lines.

Methods

- All cell lines used were from DiscoveRx Corporation and express various GPCR tagged with ProLink in cells stably expressing EA-β-Arrestin2 for the PathHunter™ β-Arrestin assay or untagged receptors in HEK and CHO cAMP Hunter™cells.
- cAMP Assays were performed using the HitHunter™ cAMP XS+ assay kit
- For PathHunter assays, 5000 cells per well were seeded in 20 µL media and incubated overnight prior to assay.
- For agonist assays, 5 µL 5x compound was added to cells and incubated at 37°C/5% CO_2 for 60-90 minutes.
- For antagonist assays, 5 µL 5x compound was added to cells and incubated at 37°C/5% CO_2 for 60 minutes, after which 5 µL 6x EC_{80} agonist was added and incubated for 60-90 minutes at 37°C/5% CO_2.
- For inverse agonist assays, cells were incubated with compound for 16 hours at 37°C/5% CO_2.
- β-Arrestin binding was detected after 1 hour room temperature incu bation with 50% (v/v) of PathHunter Detection Reagent (93-0001).
- Data was read on Packard Victor 2 or PerkinElmer ViewLux readers and analyzed using GraphPad Prism 4.

- AGTR1

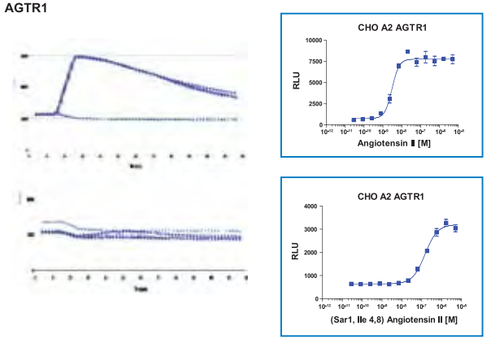

Figure 2. PathHunter Arrestin Detects Biased Ligands. CHO-K1 cells expressing β-Arrestin2-EA and AGTR1-PK were exposed to the known agonist ATII or the antagonist SII. The samples were analyzed for the calcium response or β-galactosidase activity indicating arrestin binding to the GPCR. As expected, treatment with the agonist ATII elicited a robust response in the calcium and β-Arrestin2 assays. β-Arrestin2 is also recruited in response to the SII compound but the ligand does not activate the G-protein mediated calcium response.

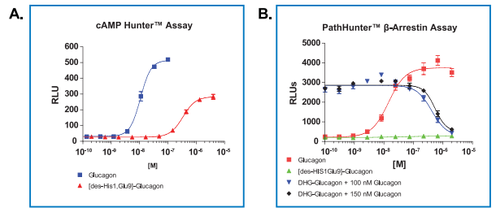

Figure 3. Biased Agonism of the Class B Receptor, GPCR. cAMP Hunter™ cells expressing unmodified GCGR (**A**) or the PathHunter™ β-Arrestin GCGR assay (**B**) tested for responses to the full agonist, glucagon, or the partial agonist, des-his1,glu9-Glucagon.

As expected the cAMP Hunter assay shows a robust cAMP increase in response to glucagon. A full agonist response to glucagon is also observed in the arrestin binding assay. The des-his1, glu9-Glucagon compound elicited a partial agonist response in cAMP, but performed as an antagonist in the β-Arrestin assay.

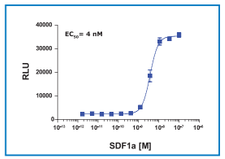

Figure 4. CXCR7 is an Arrestin-biased receptor. CXCR7 Case Study. Originally thought to be a "decoy" receptor, CXCR7 does not activate any second messenger signaling pathway and has no chemotaxis response. However, it does show robust arrestin recruitment in the PathHunter assay.

A.

Target GPCR	Common Name	Reference Ligand	cAMP	Arrestin	Binding
ADCYAP1R1	PAC1	PACAP-38	0.48 nM	29.7 nM	14 nM
ADORA1	A1	Cyclopentyladenosine	40.6 pM	2.7 nM	436 nM
ADORA2B	A2B	NECA	3.8 nM	1300 nM	570 nM
ADRB2	B2AR	Isoproterenol	360 pM	21.2 nM	380 nM
CCR1	CC1	CCL3/MIP1a	3.2 nM	1.4 nM	0.056 nM
CCR10	GP2	CCL27	10.3 nM	20.4 nM	
CCR5	CC5	CCL3/MIP1a	1.8 nM	0.69 nM	14 nM
CCR6	CC6	CCL20	52.1 pM	102pM	6 nM
CCR7	CC7	CCL19	0.9 nM	14.9 nM	6 nM
CNR1L	CB1	CP55940	164 nM	3.2 nM	2.9 nM
CNR2	CB2	CP55940	24.5 nM	4.8 nM	2.5 nM
CRHR1	CRF1	Sauvagine	1.8 nM	13 nM	0.11 nM
CRHR2	CRF2	Sauvagine	2.1 nM	32 nM	0.21 nM
CRTH2	DP2	Prostglandin D2	1.9 nM	264 nM	23 nM
CXCR1	IL8RB	Interleukin-8	1.8 nM	1.3 nM	2 nM
CXCR2	IL8RB	Interleukin-8	4.5 nM	2.6 nM	2 nM
CXCR3	CXC3	CXCL11	16.8 nM	17.5 nM	0.069 nM
EDG1	S1P1	S1P	1.8 nM	30 nM	50 nM
FPRL1	ALX	WKYMV	0.41 nM	2.7 nM	
GALR1	GAL1	Galanin	18.0 nM	32 nM	
GCGR	GC0	Glucagon	31.7 nM	15.3 nM	3 nM
GLP1R	GLP1	Exendin-4	96.4 pM	2.2 nM	
HRH2	H2	Histamine	48.6 nM	9040 nM	
MC3R	B4	Leukotriene B4	723.1 nM	211.7 nM	
MC3R	MC3	aMSH	26.0 nM	17.0 nM	9.4 nM
MC4R	MC4	aMSH	212.4 nM	69 nM	9.2 nM
MC5R	MC5	Melanotan II	136.1 nM	2.2 nM	0.9 nM
MCHR1	MCH1	MCH	46.4 nM	61 nM	0.043 nM
PTHR2	PTH2	TIP39	0.13 nM	3.2 nM	
SCTR	SEC	Secretin	107.8 pM	2.8 nM	
SSTR4	SRIF2B	Somatostatin 14	0.61 nM	0.73 nM	

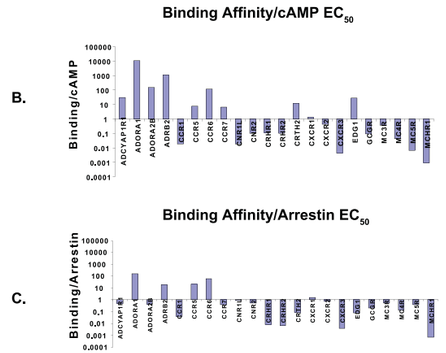

Figure 5. Comparison of Binding Affinity, cAMP response, and Arrestin recruitment. The ligand potency of 32 cAMP Hunter™ cell lines were compared against the corresponding 32 PathHunter™ β-Arrestin cell lines using reported binding affinities as a benchmark for both assays (**A**). To graphically represent the three types of measurements, the binding affinity (K_d) for each ligand was divided by the EC_{50} from the corresponding assay for cAMP (**B**) and Arrestin (**C**). Overall the PathHunter Arrestin cell lines have less variation and are more similar to the binding values than the cAMP measurements.

Summary

The discovery of arrestin-biased ligands presents a new parameter for compound characterization. During the course of these studies we observed:

- PathHunter™ β-Arrestin assay accurately identified SII as a perfectly arrestin biased ligand of AGTR1
- des-his1-glu9-Glucagon acts as a partial agonist in cAMP assays but behaves as an antagonist in arrestin recruitment
- CXCR7 recruits β-Arrestin but fails to activate second messenger assays and represents a perfectly biased receptor
- PathHunter β-Arrestin EC_{50} values more closely parallel binding affinities than cAMP
- Relationship between binding affinity, $EC_{50}$of second messenger, and EC_{50} of β-Arrestin for specific ligands is dependent on the target and the compound

DiscoveRx
www.discoverx.com