GPCR in cardiovascular research: The relevance of camp and IP-1 second messenger assays

Abstract

GPCRs are key players in cell signaling and a cornerstone of research in cardiovascular disorders and diseases (CVDs). This note compiles published examples of cardiovascular related studies in which cAMP and IP-One HTRF second messengers assays were successfully used to monitor Gi/s and Gq activity and allowed for a better understanding or addressing of CVDs.

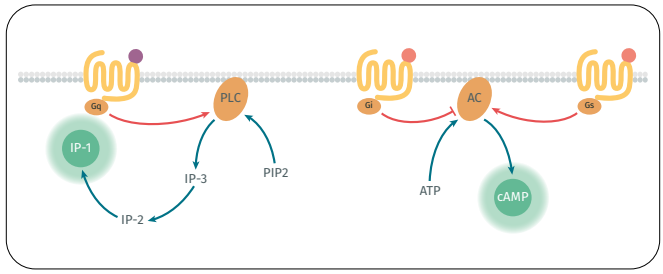


Figure 1: IP-1 and cAMP second messengers in Gq or Gi/Gs coupled GPCRs respectively

GPCRS involved in cardiovascular function

Table 1 : GPCRS involved in cardiovascular function

Gq coupled receptors		Gi/o coupled receptors	Gs coupled receptors
5-HT	VIP	5-HT	5-HT
Acetylcholine	Prostalglandin D2, E2, F2a	Acetylcholine	Adrenaline
Adrenaline	Prostacyclin	Adrenaline	Apelin
Dopamine	Platelet activation factor	Histamine	CCL3
Histamine	Leukotriene C4, D4	Noradrenaline	CCL4
Adrenomodulin	Adenosine	Angiotensin-II	CCL5
Apelin	ADP, ATP, UTP	Endothelin-1	Chemerin
Bradykinin	17b-Estradiol	Kisspeptin	Endothelin-1
CGRP	Sphingosine-1-phosphate	Neurokin A	Neuropeptide Y
Endothelin-1	Serine protease	Neuromedin U	Somatostatin
Ghrelin	Thrombin	Urotensin-II	Prostalglandin

Gq coupled receptors	Gi/o coupled receptors	Gs coupled receptors
Motilin	Vasopressin	ADP, ATP
Neurotensin	Prostalglandin E2	Adenosine
Nocipeptin	Thromboxane A2	Sphingosine-1-phosphate
Relaxin	Leukotriene D4	Thrombin
Substance P	ADP, ATP, UDP	
Urocortins	Adenosine	
Urotensin-II	Sphingosine-1-phosphate	
Vasopressin	Thrombin	

IP-One assay characterizes ligands and receptors

Case of the angiotensin II type 1 receptor

Violin J.D, et al. (2010) JPET 335:572-579
Siddiquee, et al. (2011) BJP 168(5):1104-17

β-Arrestin is a multifunctional signaling protein that mainly acts upon GPCRs by desensitizing G protein signaling, triggering the receptor internalization and promoting G protein-distinct signaling pathways. Due to their potency to inhibit broad G protein signals and activating much more straightforward transduction cascades, β-Arrestins offer attractive opportunities to develop therapeutics less susceptible of carrying adverse effects than usual GPCR targeted drugs. the Angiotensin II type 1 receptor (AT1R) is one the most studied GPCRs that exhibits all three mentioned effects upon β-Arrestin binding.

n a 2010 study, Violin *et al.* aimed to develop new β-Arrestin biased ligands for the AT1R and evaluate whether such ligands would exhibit different *in-vivo* effects from unbiased ligands. They custom-synthesized peptides based on the angiotensin II (SII) and evaluate their respective ability to alter β-Arrestin recruitment and G signaling.

To that end, they used the HTRF IP-One assay to assess the selective activation or inhibition of Gq coupled GPCRs and monitored the G-signaling inhibitory potential of their candidates.

Table 2: β-Arrestin recruitment and IP-1 accumulation (G-signaling activity) IC50 analysis for Angiotensin II and two novel ligands. Data from Violin J.D *et al.*, 2010, JPET 335:572-579

Ligand	β-Arrestin recruitment IC50	G protein activation (IP-1 accumulation) IC50
Angiotensin II	9.7 nM	1.1 nM
TRV120023	44 nM	No activation
TRV120027	17 nM	No activation

The results allowed for the identification of TRV120027 as a potent β-Arrestin biased ligand, susceptible of inhibiting the G-signaling function of the AT1R while efficiently promoting β-Arrestin recruitment and related effects (receptor internalization, distinct transduction signal). More specifically, they found this ligand reducing MAP signaling downstream of G-proteins activation, while increasing cardiac contractility, which they suggest could prove beneficial in heart failure syndromes.

Pushing the understanding of the Angiotensin II type 1 receptor (AT1R) further, Siddiquee *et al.* sought to study the known yet not explained inhibition of AT1R by the Apelin Receptor (APJ) (2012).

Using the same β-Arrestin recruitment and HTRF IP-One assay for G-signaling monitoring approach they were able to quantify and conclude on the AT1R G-signaling inhibition upon Apelin-induced heterodimerization of AT1R and APJ, which adds relevance to this interaction in the context of cardiovascular diseases pathogenesis.

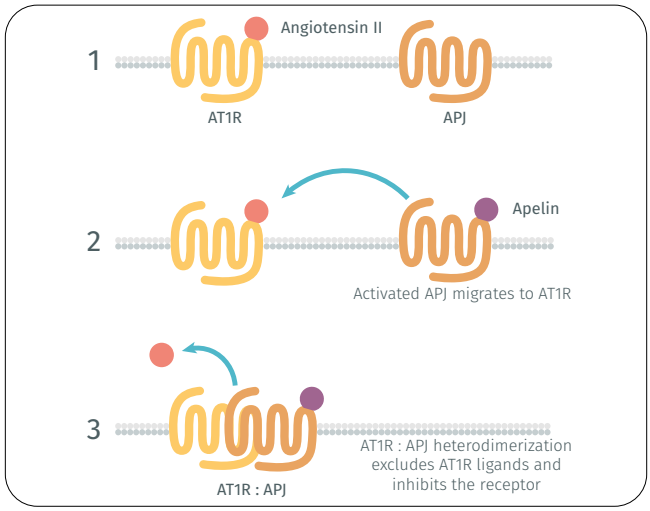


Figure 2: Apelin-dependent activation of APJ triggers AT1R:APJ heterodimerization which inhibits AT1R. Adapted from Siddiquee *et al.*, 2011, BJP 168(5):1104-17

Screening for drug candidates with the camp assay

Case of the relaxin RXFP1 receptor

Chen C.Z, et al. (2013) J Biomol Screen 18(6): 670-677

Relaxin is a hormone playing many roles in body mechanisms, including regulation of the cardiovascular function in which it activates the nitric oxide pathway. It binds to receptors RXFP1 or RXFP2, both members of the relaxin/insulin-like family of GPCR peptide receptors, and its signal transduction is effected through cAMP levels modulations.

In a 2013 study, Chen *et al.* sought to develop a quantitative HTS platform suited for the screening of inhibitors of the RXFP1 receptor. They based their work on the HTRF cAMP assay, which they optimized and validated in a 384-well plate format assay, before scaling it up to a 1536-well plate screening format.

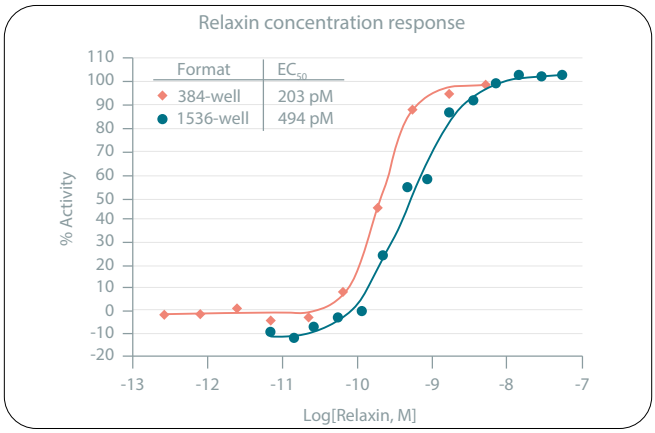


Figure 3: Assay miniaturization and validation – (a) Dose response analysis of RXFP1 activity to relaxin, (b) HTRF signal from DMSO, relaxin and Forskolin, (d) Scatter plot of the ratiometric signal from a 1536-well DMSO plate.

Using this HTRF cAMP assay, they were able to screen over 365,000 compounds and narrow them to 434 hits, two of which later demonstrated selectivity against the RXFP1 receptor.

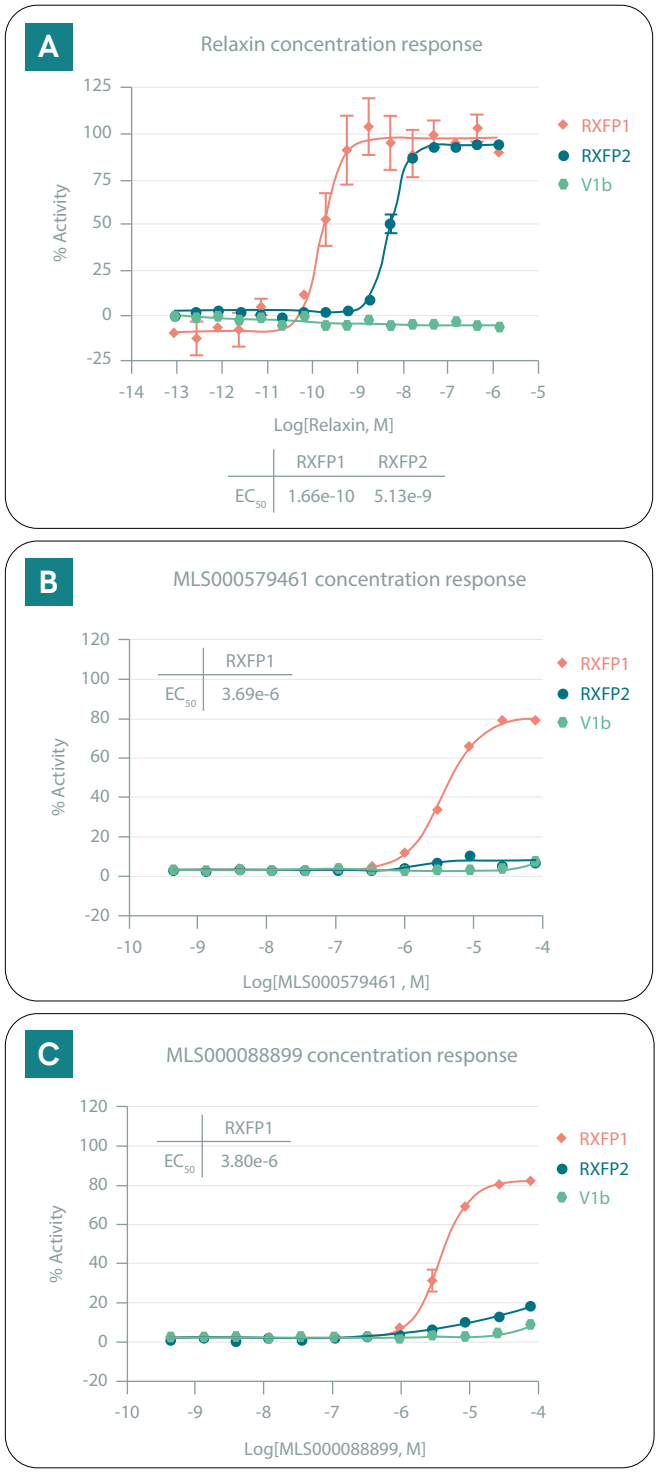


Figure 4: HTRF cAMP response to (a) relaxin, (b) MLS000579461 and (c) MLS000088899

Investigate the cardiac function with camp assays

Atrial natriuretic peptide in ventricular development

Hotchkiss A, et al. (2015) Am J Physio Cell Physiol 308: C557–C569

Early stages of atrial and ventricular chambers differentiation in embryos are driven by Atrial Natriuretic Peptides (ANPs) signaling through ANPR receptors that modulate cGMP (increase) or cAMP (decrease) levels. While such ANP levels remains in the atria in later embryogenesis, they decrease in the ventricles and it is unknown whether they remain expressed and/or active in developing ventricles.

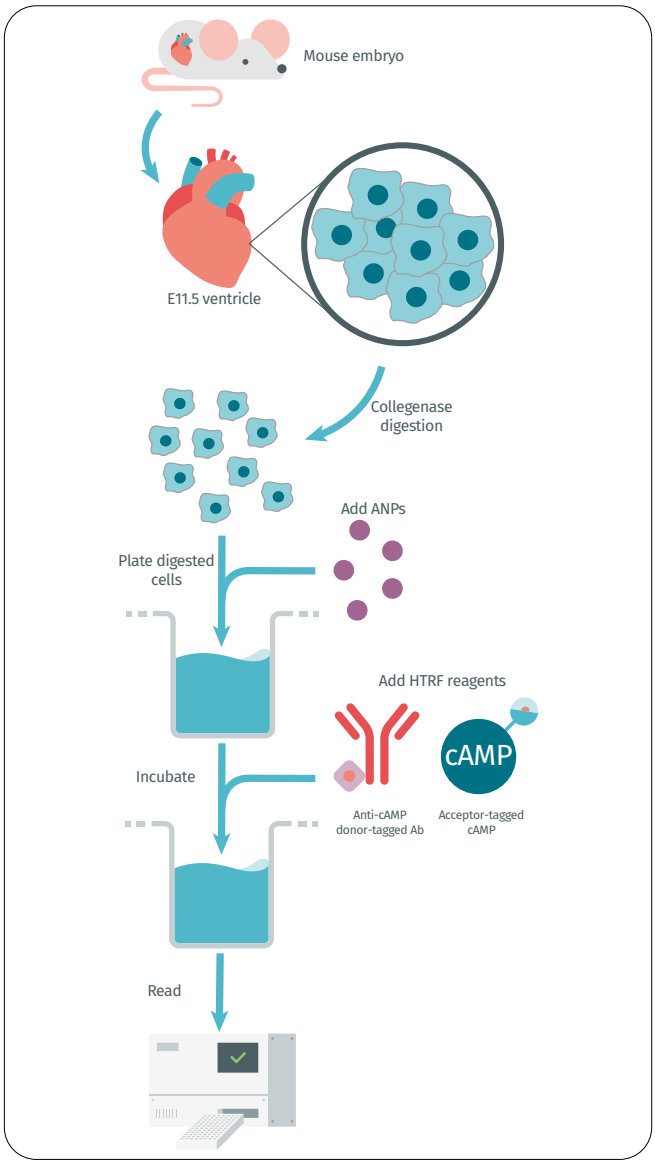


Figure 5: Assay principle – Embryonic ventricle tissues are digested and plated, then stimulated with ANPs. After incubation, the HTRF assay reagents are added to the mix and results are read.

In 2015, Hotchkiss *et al.* investigated the ANP signaling activity in embryonic ventricles to characterize the role of ANPs in cardiac progenitors and/or cardiomyocytes differentiation. They used the HTRF cGMP and cAMP assay kits to monitor cGMP and cAMP levels in digested E11.5 embryo ventricle cells (figure 5).

The assays allowed them to determine basal levels of cGMP and cAMP, and assessed the absence of effect of ANPs (1-100ng/mL) on cAMP levels. This finding was strengthened in another assay in which isoproterenol-stimulated cAMP levels were not lowered by high doses of ANPs (100 or 1000 ng/mL). cGMP levels, however, proved sensitive to ANPs, leading the authors to conclude on the activity of the guanylyl cyclase related ANPRs in ventricles at this stage, while the adenylyl cyclase related ANPRs activity could not be confirmed.

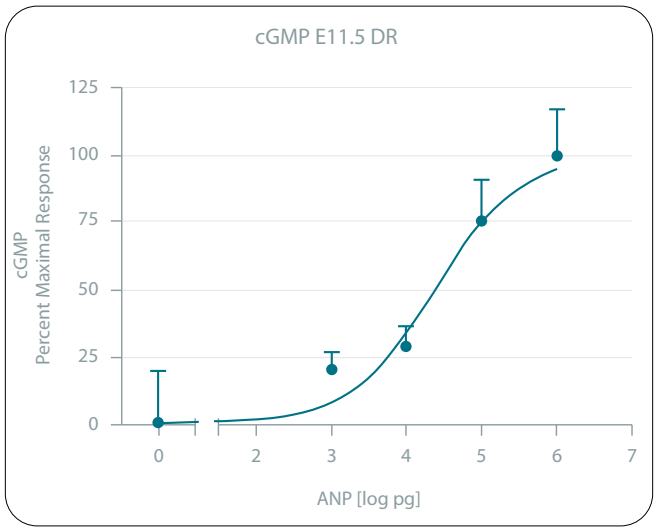
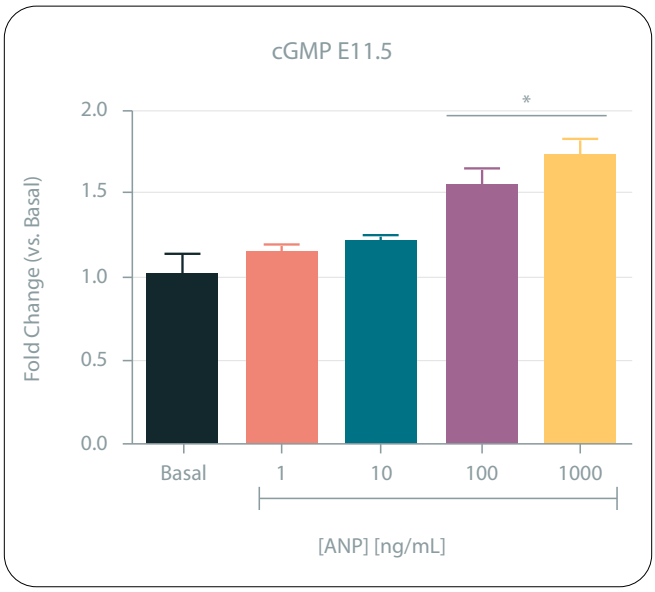


Figure 6: Dose/response analysis of cGMP production in E11.5 ventricular cells following incubation with ANPs.



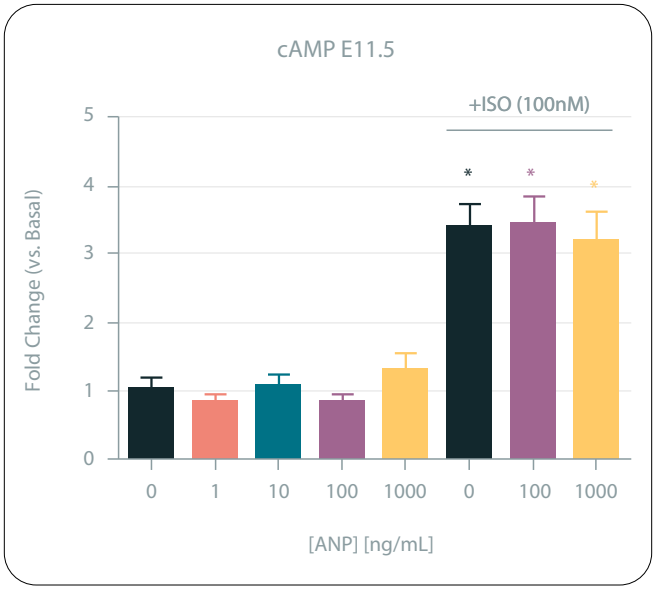


Figure 7: cGMP and cAMP production in E11.5 ventricular cells following incubation with ANPs. (a) Fold change to basal level of cGMP, (b) Fold change to basal level of cAMP

Conclusion

cAMP and IP-1 are key second messengers in cardiovascular research and empower scientists with the ability to monitor GPCR activity with significant specificity and efficiency. Publications in this note provide an insight into how HTRF assays can be relevant and provide valuable results for cardiovascular research, whether it is fundamental or therapy oriented.

Works cited

A. Hotchkiss, T. F.-N. (2015). Atrial natriuretic peptide inhibits cell cycle activity of embryonic cardiac. Am J Physiol Cell Physiol.

C.Z. Chen, N. S. (2013). Identification of small molecule agonists of human relaxin family receptor 1 (RXFP1) by utilizing a homogenous cell-based cAMP assay. J Biomol Screen.

J.D. Violin, S. D. (2010). Selectively engaging beta-Arrestins at the Angiotensin II Type 1 receptor reduces blood pressure and increases cardiac parformances. The Journal of Pharmacology and Experimental Therapeutics.

K. Siddiquee, J. H. (2011). the apelin receptor inhibits the angiotensin II type 1 receptor via allosteric trans-inhibition. British Journal of Pharmacology.

HTRF assays cited

Product	Tests	Cat. No.#
cAMP – HiRange kit	1000	62AMPEB
cAMP – Gi Dynamic kit	1000	62AM9PEB
cAMP – Gs Dynamic kit	1000	62AM4PEB
IP-One – Gq kit	1000	62IPAPEB

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