β-ARRESTIN-2 RECRUITMENT TO MONITOR HISTAMINE H4 RECEPTOR ACTIVATION OVER TIME

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Introduction

Histamine is a biogenic amine which synthesised by the pyridoxal phosphate-containing L-histidine decarboxylase from histidine. It is stored as intracellular vesicles in hematopoietic cells, including mast cells, eosinophils, and T cells. This indicates that they are playing a significant role in inflammatory response.[1] Histamine exerts its effect onto histamine receptor which is a family of GPCRs. There are 4 distinct histamine receptor subtypes: H1, H2, H3 and H4 receptors (H4R). Recently, the histamine H4R has been elucidated to also signal via β-arrestin-2 recruitment pathway apart from G-protein activation. β-arrestin-2 is recruited to H4R following its activation and phosphorylation.[2] This uncouples G-proteins from H4R, promotes their internalisation and results in receptor desensitisation.

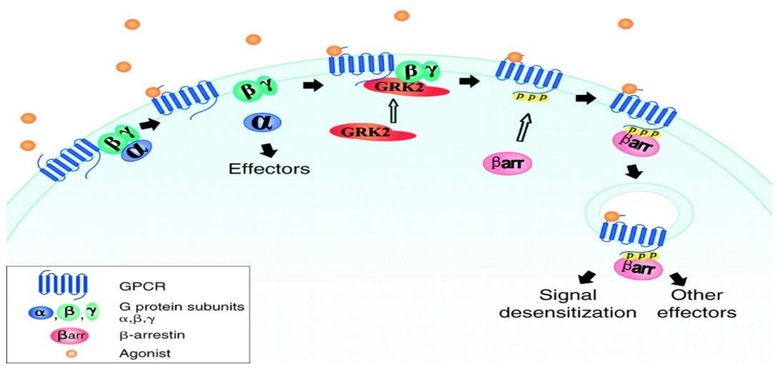
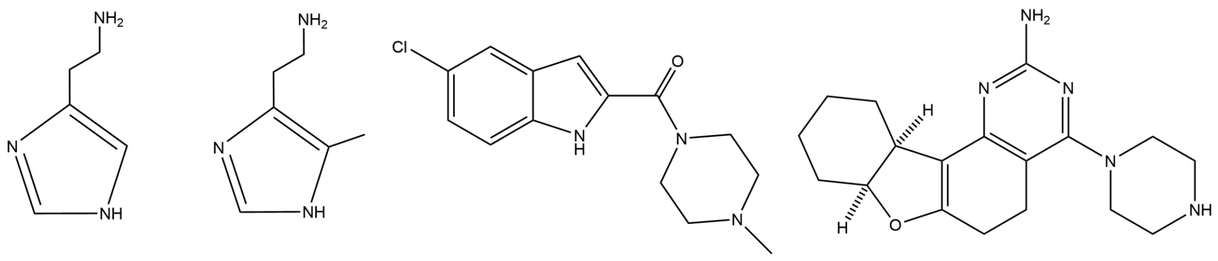


Figure 1. Basic Scheme of negative feedback β-arrestin-2 and endocytosis of GPCR.[3]

Objectives

The aim of this study is to fully categorise a range of ligands given at the histamine H4R in terms of their potency and efficacy for recruiting β-arrestin-2 to the receptor. In addition, the rate of β-arrestin-2 recruitment will be determined for different classes of compound.



Full agonist Histamine | **Full agonist** 4-methyl histamine | **Partial agonist** JNJ7777120 | **Antagonist** A987306

Figure 2. The compound series investigated in this study.

Methods

U2OS-H4R cell culture and DiscoveRx PathHunterTM β-arrestin-2 assay (DiscoveRx) were performed to monitor β-arrestin-2 recruitment. DiscoveRx uses β-galactosidase enzyme fragment complementation to measure the recruitment of β-arrestin-2 to a GPCR after phosphorylation and activation. Recombinant U2OS-H4R cells were engineered to co-express the ProLink™ (PK) tagged GPCR and the Enzyme Acceptor (EA) tagged β-Arrestin-2. Cells were then incubated with full agonist (Histamine and 4-methyl histamine), partial agonist (JNJ7777120) and antagonist (A987306) at concentration of 10^{-5}M-10^{-9}M for up to 4 hours. Upon receptor activation by ligands, β-arrestin-2 was recruited to H4R, this resulted in interaction of two β-galactosidase enzyme fragments (EA and PK). Thus, it produced a functional enzyme, whose activity can be measured by addition of hydrolysable substrate and generation a chemiluminescent signal. The luminescent signal was then read by EnVision plate reader.[3] Respectively, concentration-response curves were plotted using Graphpad Prism 6.

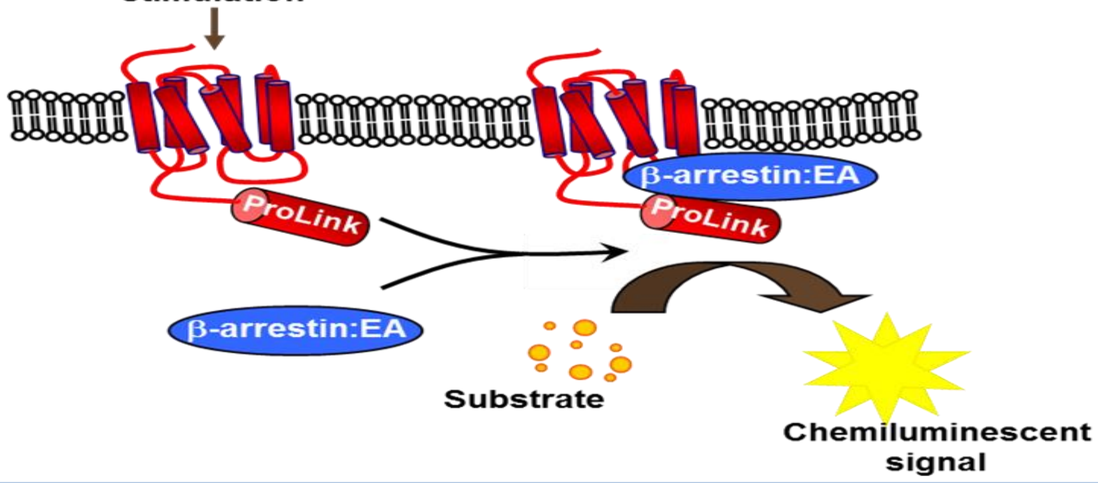
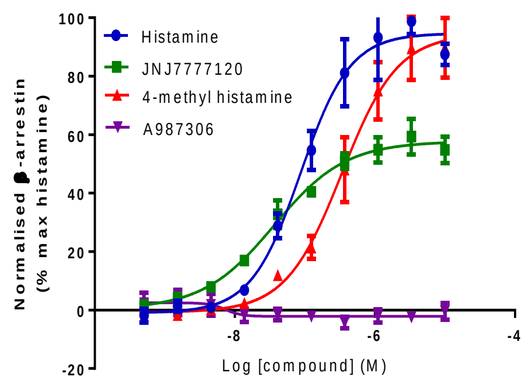


Figure 3. Principles of DiscoveRx PathHunterTM β-arrestin-2 assay. Upon activation by agonist, β-arrestin-2:EA protein is recruited to histamine H4R:ProlinkTM protein to form a functional enzyme. By adding hydrolysable substrate to enzyme, chemiluminescent signal is produced.

Key Results



Compounds	β-arrestin-2	
	pEC_{50}	Efficacy (%)
Histamine	7.0 ± 0.1	100
JNJ7777120	7.5 ± 0.1*	57.8 ± 5.0**
4-methyl histamine	6.4 ± 0.1*	96.1 ± 12.2
A987306	ND	ND

Figure 4. Ligand-mediated β-arrestin-2 recruitment in U2OS-H4 cells. Concentration-dependent increases in β-arrestin-2 recruitment were assessed after stimulation with the indicated concentrations of ligands for 1hr. The data is normalised at 10μM Histamine at time 1hr.

Table 1. Efficacy (%) and potency (pEC_{50}) of agonists in β-arrestin-2 recruitment are expressed as mean ± S.E.M, n=3 and performed in duplicate. Significant differences from histamine were quantified by one-way analysis of variance (ANOVA), followed by Dunnett's post-test analysis. **P<0.01, *P<0.05.

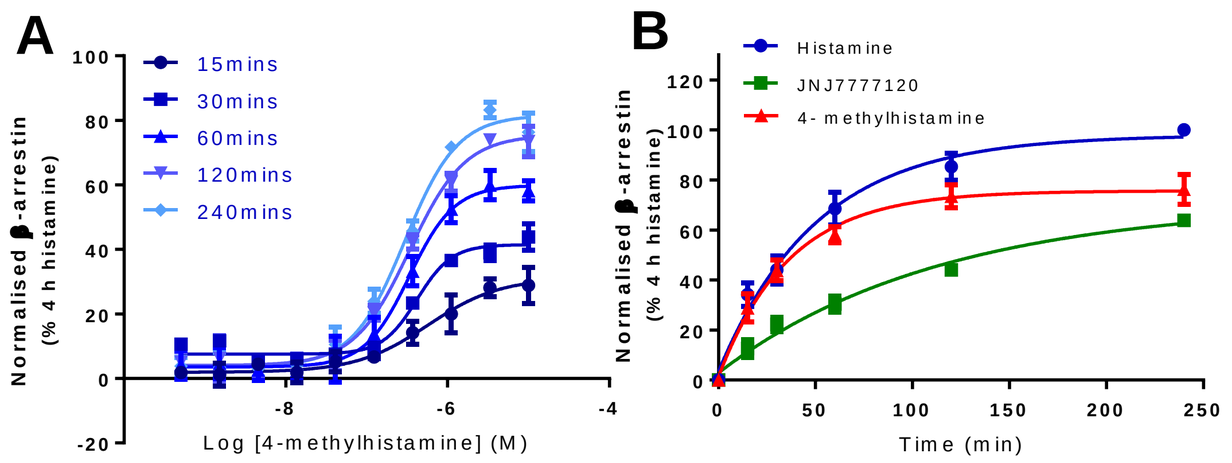


Figure 5. (A) The time course of concentration dependence increase of β-arrestin-2 recruitment of 4-methyl histamine **(B)** Comparison of rate of β-arrestin-2 recruitment between 10μM 4-methyl histamine, 10μM JNJ7777120 and 10μM Histamine over 4 hours. Data are expressed as mean ± S.E.M, n=3 and performed in duplicate. The data is normalised at 10μM Histamine at time 4 hour.

Compounds	β-arrestin-2 (4 hr time course)	
	Half-life (min)	Efficacy (%)
Histamine	32.9 ± 6.7	100
JNJ7777120	83.5 ± 22.2	63.9 ± 1.1
4-methyl histamine	25.3 ± 2.8	76.1 ± 4.7

Table 2. Efficacy (%) and half-life (min) of agonists in β-arrestin-2 recruitment over 4 hours. Data are expressed as mean ± S.E.M, n=3 and performed in duplicate. The data is normalised to 10μM Histamine at time 4 hour.

- Histamine and 4-methyl histamine showed the greatest efficacy in β-arrestin-2 recruitment (Table 1).
- The efficacy of JNJ7777120 was significantly lower than that of histamine. The order of efficacy among ligands was Histamine > 4-methyl histamine > JNJ7777120 (Table 1).
- 4-methyl histamine significantly showed the lowest potency for H4R among all compounds with pEC_{50} values of 6.4 ± 0.1 (Table 1).
- JNJ7777120 had the longest half-life in β-arrestin-2 recruitment over 4 hours (Table 2).
- The efficacy of β-arrestin-2 recruitment of 4-methyl histamine was lower than maximal response of histamine over 4 hours (Table 2).

Conclusion and Future Implications

- 4-methyl histamine and histamine are regarded as full agonist due to their similar efficacy profiles.
- From these data, there appears to be a relationship between efficacy and rate of β-arrestin-2 recruitment. The higher efficacy ligands histamine and 4-methyl histamine have faster rate of β-arrestin-2 recruitment. In contrast, the lower efficacy ligand JNJ7777120 has a slower rate of β-arrestin-2 recruitment.
- Future work could focus more on 4-methyl histamine as there was bigger error bar on the concentration-response curve and only had single concentration of histamine was normalised to over 4 hour ,resulted in a more variable response. Modification to the structures of partial agonist could be done to develop therapeutic agents for H4R related diseases over a neutral antagonist for long term treatment.

Acknowledgements and References

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