Point substitutions in G Protein-Coupled Receptors

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Introduction

- G protein-coupled receptors (GPCRs) are proteins that are important in physiological regulatory processes within the body, and for this reason are important drug targets
- When bound to an agonist, such as neurotransmitters or hormones, the receptor adopts an active state to allow these biochemical pathways to occur
- Mutations can arise within the receptor that affect its ability to bind its agonist
- **Purpose:** To test whether mutations within the sodium ion binding pocket, an allosteric site, play a role in agonist-induced receptor activation
- **Hypothesis:** If mutations are made within the sodium ion binding site, then there will be an increase in agonist-induced receptor activation due to the loss of a negative allosteric effect

Methods

- A GloSensor cAMP assay was used to measure luminescence, which was a direct output of receptor activation
- HEK293H cells were co-transfected with mutant and wildtype receptors, as well as a GloSensor plasmid
- Agonist binding encouraged the production of cAMP, which when present with luciferin, caused luminescence to occur

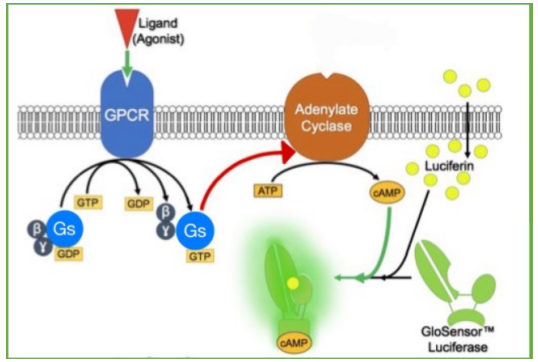

Figure 1. GloSensor Assay. Luminescence from luciferase activity shows GPCR activity (Wang et al.)

Results

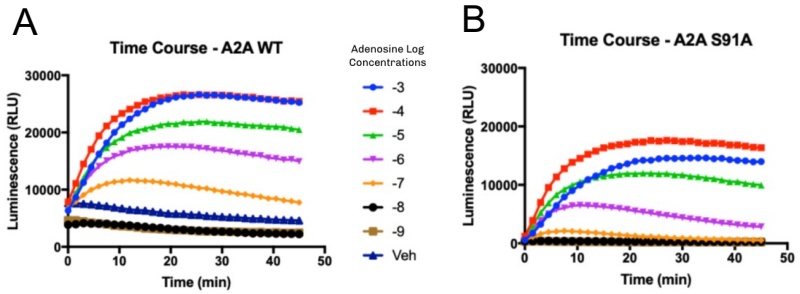

Figure 2. Spontaneous activity of wildtype receptor (A) and mutant receptor S91A (B) in the form of a time course graph. Luminescence output over time is a direct measurement of agonist-binding. It is calculated using the slopes of the data. Activity is reduced in S91A.

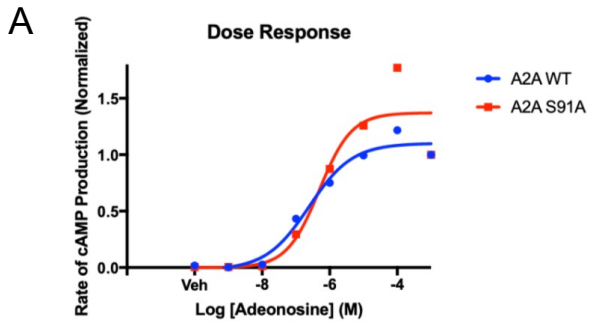

Figure 3A. The rate of cAMP production as a function of adenosine concentration, normalized to 1.0. WT response is the blue curve while mutant response is the red curve. There is no significant difference between the two curves.

Discussion

- S91A remained active, indicating that there is still agonist-induced activation that occurs
- There was reduced spontaneous activity when comparing the two time course graphs
- The dose response curve show similar responsiveness between the wildtype and mutant receptor

- In summary, mutations with the sodium ion binding site play a role in the ability of the agonist to bind the receptor
- Our findings go against those found in literature as well as our original hypothesis, as there was a decrease in agonist-induced receptor activation, instead of an increase
- More research must be completed to fully understand this concept

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