The programmable activation of the b2AR

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Introduction and Background

Aptamer

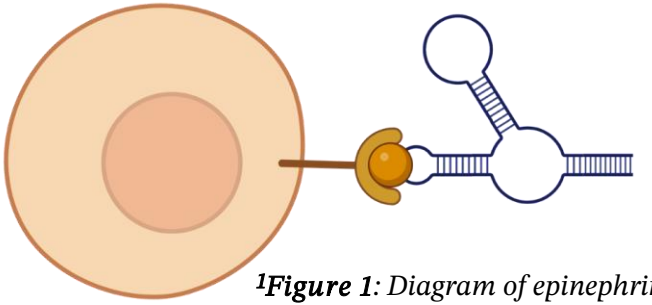
- Short Single stranded DNA or RNA molecules
- Can specifically bind to target molecules with high affinity and specificity

B2AR

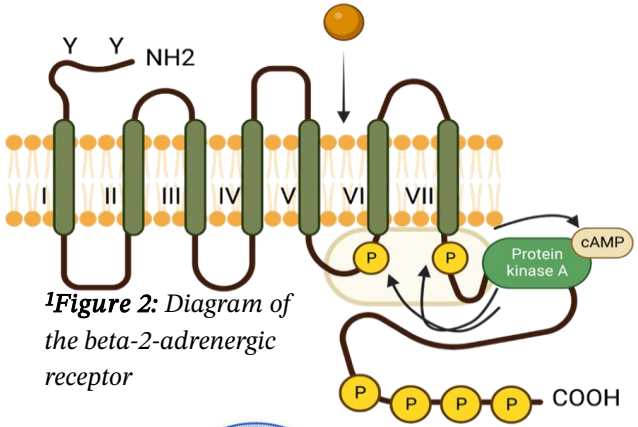
- subclass of G protein-coupled receptors or GPCRs
- play a crucial role in many physiological pathways- bronchodilation, vasodilation

Cell Internalization

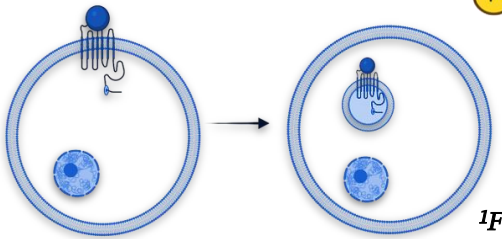
- When an agonist successfully bing to b2ar, the receptor internalized



[1]***Figure 1***: *Diagram of epinephrine-aptamer complex bound to cell receptor*



[1]***Figure 2:*** *Diagram of the beta-2-adrenergic receptor*

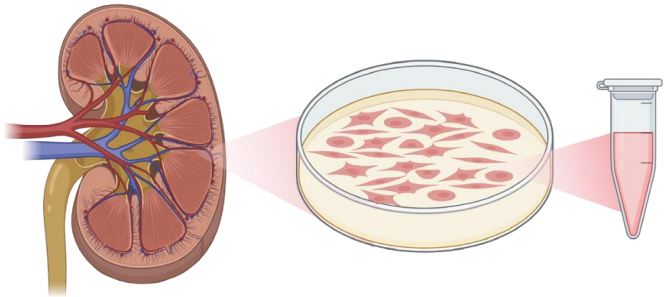


[1]***Figure 3:*** *Diagram depicting cell internalization*

Introduction and Background Continued

Human Embryonic Kidney Cells

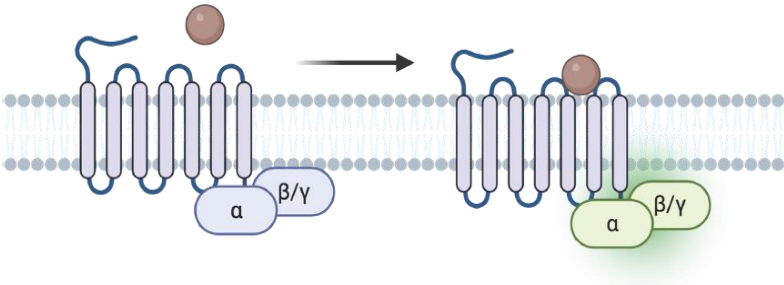
- HEK 293/293T cells are derived from human embryonic kidney transformed with adenovirus 5 DNA
- HEK 293 cells are highly amenable to transection.
- They express the b2AR on their cell surface[5]



[1]***Figure 4:*** *Diagram of W9 HEK adherent cells*

Epinephrine

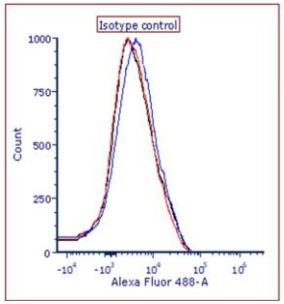
- Epinephrine or adrenaline is a catecholamine and a mono amine that is released from the adrenal medulla in response to stress.
- Receptors for epinephrine are called adrenergic receptors. Epinephrine stimulates the alpha and beta subtype of adrenergic receptors[4]

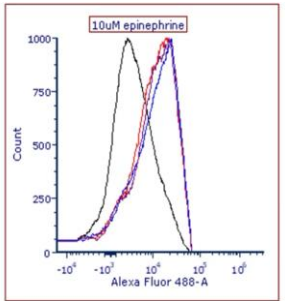


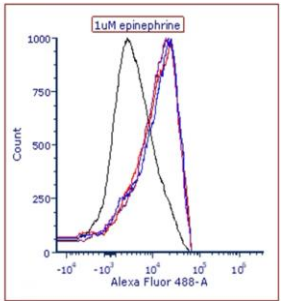
[1]***Figure 5:*** *Depiction of G protein activation due to agonist binding*

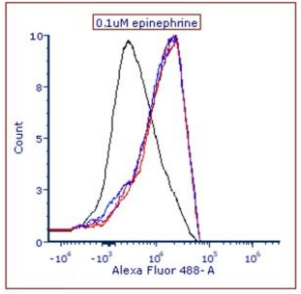
Methods and Results

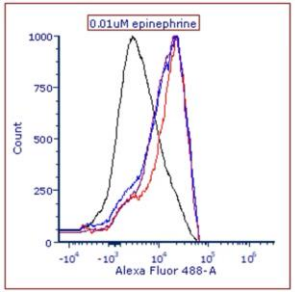
- Analysis of cell internalization with different concentrations of epinephrine.
- 25uL of the diluted rabbit IgG isotype and IgG antibody tagged with Alexa Fluor 488 were utilized.
- The change in fluorescent intensity was measured using flow cytometry and the data obtained was analyzed with FCS Express software.
- **Results:** Epinephrine activates b2AR in W9 cells. As a result,the receptor is internalized in a dose-dependent manner, with an EC50 value of 8nM.

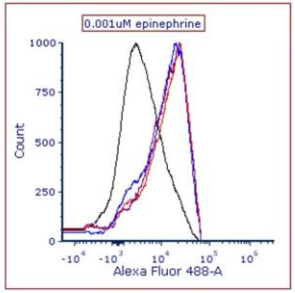


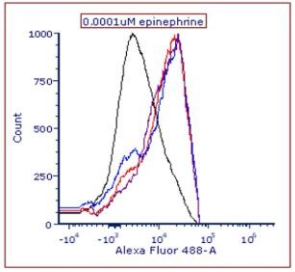


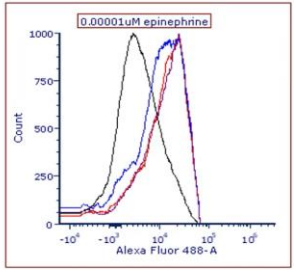


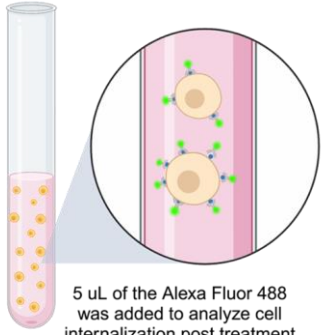




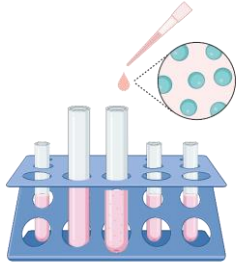




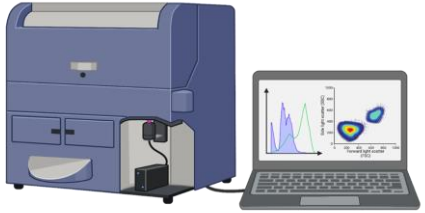




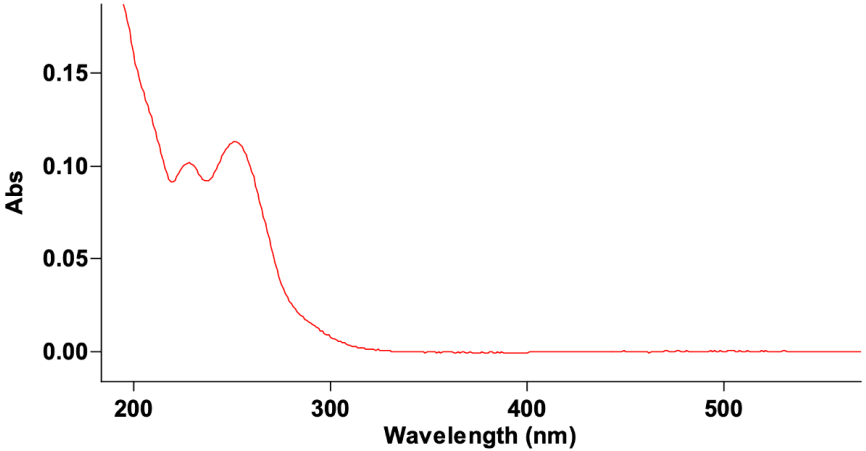
5 uL of the Alexa Fluor 488 was added to analyze cell internalization post treatment



Cells were treated with different concentration of the epinephrine agonists

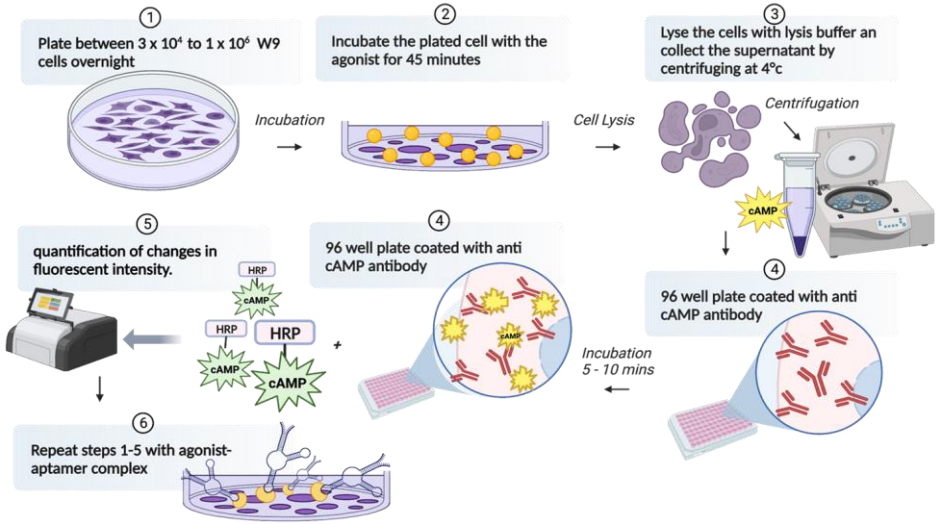


Methods and Results



- For DNA and RNA, the maximum light absorbtion occurs at or around 260 nm.
- The absorption peak for the epinephrine aptamer is around 260
- This indicate that the base pairs of the epinephrine aptamer is in fact DNA or RNA based

- comparative ELISA fluorometric assays was used to monitor epinephrine mediated b2AR activation.
- cAMP is released in response to b2AR activation.Thus, the b2AR activation in response to epinephrine with or without aptamer will be measured.



Conclusion and Further Research

- The modified HEK cell line does in fact have the b2AR receptor
- Epinephrine is able to successfully bind to the b2ar and induce internalization
- Epinephrine aptamer is DNA or RNA based
- The use of epinephrine-specific aptamers is a desirable method of masking or triggering cAMP.
- Future work is aimed at engineering aptamer-based programmable sensors anchored onto b2AR-expressing cells that sense the release of epinephrine in response to stress and other physiological stimuli.
- cAMP was not performed in time

Acknowledgement

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