Discerning the Efficacy of Potential Non-Opioid Pain Drugs Using cAMP Analysis

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I. Introduction

G protein-coupled receptors (GPCRs) are a relatively new discovery in molecular biology and have been fundamental for the development of new drug therapies. GPCRs are receptors within the outer membrane of a cell that trigger intracellular reactions. A ligand binds to this protein receptor, causing a conformational change that activates the production of cAMP - a molecule responsible for various intracellular reactions.

Regulation of cAMP can lead to the inhibition or enhancement of intracellular processes. Such regulation already occurs naturally via GPR171, which is greatly involved in morphine-induced pain-relief. A newfound ligand, MS0015203, activates GPR171 and is capable of enhancing morphine's pain-relieving properties; making it a potential treatment for chronic pain.

MS0015203 has been given one modification for each ligand. In total, there are eight modified ligands. This research will indicate which modification has the most potential as new pain-therapies.

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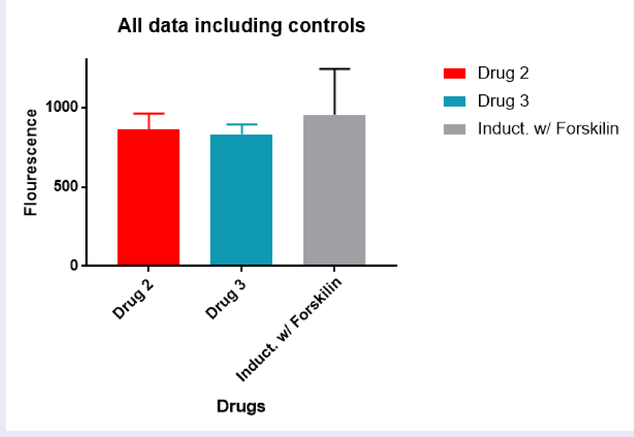
II. Methods

Brain cells possessing GPR171 were cultured with DMEM IX + 10% FBS + 1% Penicillin. A Standard Curve was created to correspond fluorescence with cAMP concentration. Afterwards, cells were:

- Treated with each ligand and each control in triplicates.
- Controls were made of induction buffer with and without forskolin - a cAMP inhibiting compound.
- Each well was treated with reagents from the cAMP-Glo™ kit to create fluorescence
- SpectraMax measured the cells to test levels of fluorescence.
- Cell cAMP concentration was determined using cAMP Standard Curve

Figure 1 – cAMP inhibition capability



III. Expected Results

The unmodified ligand MS0015203 will show a significant amount of cAMP inhibition that has been already been displayed in previous research

- Most modifications will be able to enhance the inhibitory properties of GPR171 to some extent
- Ligand modification will have a varying ability to effect cAMP production
- Modifications displaying the greatest enhancement of inhibitory GPR171 will result in the greatest ability to substantially lower concentrations of intracellular cAMP

IV. Conclusions

Presently, it has been determined that ligand #2 and ligand #3 are only slightly effective at lowering cAMP concentrations. These two ligands are actually less effective at modifying cAMP concentrations than the unmodified ligand, MS0015203. Due to culture contamination, not all ligand efficacies have been determined. Therefore, this study is inconclusive for the time being. Further research is needed to establish a significant conclusion.

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