Determination of CB1 Receptor Activity for Emerging Synthetic Cannabinoid Compounds

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Abstract

With the passing of The Synthetic Drug Abuse Prevention Act on July 9, 2012, synthetic cannabinoids were put on to Schedule I of the Controlled Substances Act based on their structure, CB_1 receptor binding and functionality. Through the years, synthetic cannabinoid structures became more and more diverse to avoid illegal classification, thus putting more emphasis onto the receptor binding and functionality characteristics. The purpose of this study is to investigate CB_1 receptor activity by measuring the ability of a known CB_1 receptor agonist to inhibit forskolin-induced cAMP levels in GH4C1 cells, and use this information to aid in synthetic cannabinoid classification.

The $\%B/B_o$ of each sample was calculated and plotted against agonist concentrations of 0.2nM, 2nM, and 4nM. Using GraphPad ©, statistical differences of $\%B/B_o$ values of the agonist concentration ranges 0.2 nM-4 nM and 2 nM-4 nM were found, and the overall goal of the study was accomplished. Future studies include method optimization and determination of receptor binding constants.

Introduction

In 2006, a new psychoactive drug known as “Spice” was quickly gaining popularity. With its roots in Western European countries and its reputation for delivering a legal high, it quickly spread to the United States. The active components were synthetic cannabinoids.

Synthetic cannabinoids are a large family of chemically unrelated structures functionally similar to the active compound of cannabis, Δ 9-tetrahydrocannabinol.

At the molecular level, these compounds bind to the same cannabinoid receptors as Δ 9-tetrahydrocannabinol in the endocannabinoid system, CB_1 and CB_2.

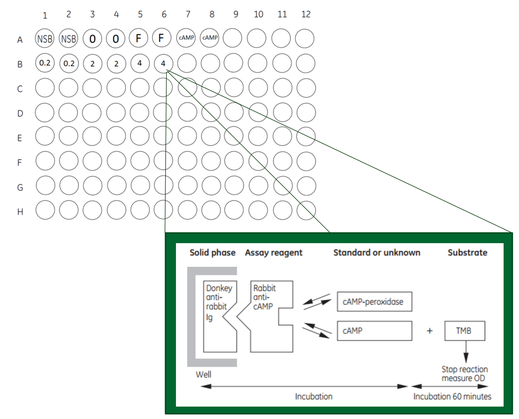
These compounds are known to inhibit the production of cAMP by negatively regulating adenylate cyclase activity.

Materials and Methods

1. Cultured GH4C1 rat pituitary cancer cells
   -Have only CB1 receptors

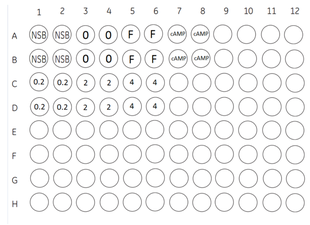
2. Transferred to 96 well plate at ~100,000 cells/well
   -Incubated overnight to acclimate to environment

3. Performed ELISA assay for cAMP
   - Amersham cAMP Biotrak EIA System ©



Materials and Methods

4. Protein Binding Normalization
   -Thermoscientific Pierce BCA Protein Assay Kit.



5. Biotek Synergy 2 Multi Mode Microplate Reader used to measure optical density at 450 nm

6. Calculated $\%B/B_o$ using the following equation

$$\left[\frac{(Sample\ or\ Std\ OD - NSB\ average\ OD)\ /\ (Sample\ protein\ concentration)}{(Zero\ standard\ OD - NSB\ average\ OD)\ /\ (Zero\ std\ protein\ concentration)}\right] \times 100$$

7. Statistical Analysis
   -GraphPad ©.

Results

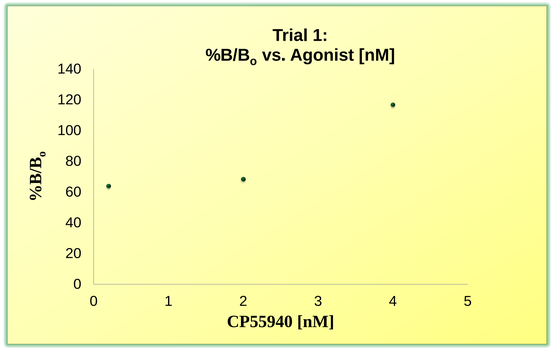


Figure 1: cAMP assay Trial 1. Shows an increasing trend of %B/Bo versus agonist concentration, which indicates a greater amount of cAMP peroxidase bound, and the more the cAMP that is inhibited.

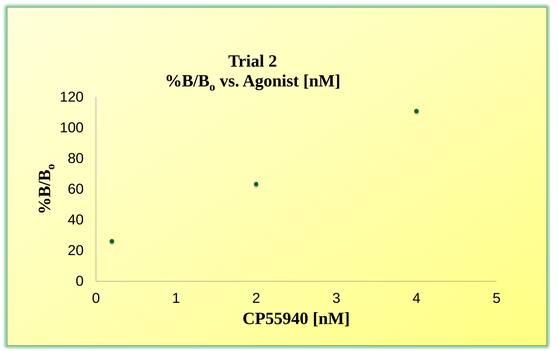


Figure 2: cAMP assay Trial 2. Shows an increasing trend of %B/Bo versus agonist concentration, which indicates a greater amount of cAMP peroxidase bound, and the more the cAMP that is inhibited.

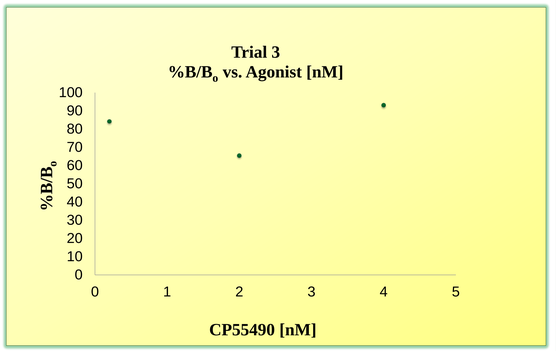


Figure 3: cAMP assay Trial 3. Shows a discrepancy for the 0.5nM concentration of CP55490, however an increasing trend of %B/Bo for the 2nM-4nM range.

Discussions and Conclusions

An ANOVA showed a significant difference between the %B/Bo values for all three concentrations of CP 55940 as indicated by an F of 5.91 ($p = 0.01$). A Tukey test was then performed to make comparisons between each of the individual concentrations.

There were significant differences between the 0.2 nM-4 nM and 2 nM-4 nM concentrations of CP 55940, however the %B/Bo values were not significant different between the 0.2 nM and 2 nM concentrations. It is possible to demonstrate CB1 agonist activity by inhibiting forskolin induced cAMP levels in GH4C1 cells.

Future studies will include method optimization with a greater sample size, with the goal of increasing the significant difference between %B/Bo values amongst the concentrations of CP 55940 to develop a model for use with compounds with unknown CB1 receptor activity.

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