**Cannabinoid-receptor interacting protein 1a (CRIP1a) effect on CB1 inverse agonists**

**I. Introduction**

Plants from the *Cannabis* genus, colloquially known as marijuana, have been used for centuries by people for various reasons, however the two predominate uses are for medical and recreational purposes1. In recent years, states throughout the US have begun the process of legalizing marijuana, the most recreationally used drug, despite it being federally illegal2. As the DEA has labeled the psychotropic chemicals of marijuana as schedule I, which is given to drugs that have a high abuse potential and have no accepted medical use, research on marijuana and how it effects humans is limited3. What is known is that phytocannabinoids, the chemicals derived from the plant, act upon the endocannabinoid system in organisms1.

The endocannabinoid system is an innate network of receptors, cannabinoid receptor (CB) 1 and CB2, and endogenous cannabinoids, which are the signaling molecules created by the body to interact with the cannabinoid receptors. The CB1 receptor is prominently expressed in the central nervous system (CNS) and much less throughout the rest of the body, while CB2 expression is more prominent in the immune system, and limited within the CNS1,5. CB1 receptors are G-protein coupled receptors (GPCRs) that perform a wide range of signaling to regulate cellular targets downstream. The C-terminal tail of GPCRs is a prominent site for protein to protein interactions, such as protein binding and post-translational modification, and have a significant amount of variability in the sequence, which provides selectivity in the protein-protein interactions4.

A novel protein isolated in 2007 by Niehaus et al. that interacts with the C-terminal tail of the CB1 receptor is the globular protein, cannabinoid receptor interacting protein 1a (CRIP1a)6. Not much is known about CRIP1a, however it is found throughout vertebrates and is mostly expressed in the brain, however it has been observed in other tissues throughout the body to a lesser degree4. While it is known that the last 9 amino acids of CB1’s C-terminus tail are the minimum required for CRIP1a to interact with CB1 receptors, it is currently unknown precisely the site on CRIP1a that binds to the C-terminus tail6. Studies performed on CRIP1a suggest that it has possibly key functions in many different physiological systems, ranging from colorectal cancers to seizure activity7.

**Figure 1. Effect of CRIP1a expression on concentration effect curves.** Results obtained from [35S]GTPS binding in cells with and without stable transfection of CRIP1a. Ligands used: 9-tetrahydrocannabinol (THC) and SR141716A (RIM). (Adapted from Ref 7)

Smith et al. (2015) did an extensive study on CRIP1a and CB1 interaction which indicated that CRIP1a is a negative regulator of CB1 function, as it inhibits constitutive CB1 receptor activity and downregulation of CB1 receptors7. Within the study a [35S]GTPS binding assay was performed with three full agonists, three partial agonists, and one inverse agonists. CRIP1a had an effect on CB1 stimulation while in the presence of the three full agonists and the inverse agonist, however CRIP1a had no effect on the three partial agonists. Figure 1 is the concentration vs CB1 stimulation curves of 9-tetrahydrocannabinol (THC), the classic phytocannabinoid, and of SR141716A, the inverse agonist, and exemplifies the reduction of the inverse agonism caused by SR141716A and the lack of change in CB1 stimulation by THC while in the presence of CRIP1a7. These findings suggest CRIP1a has a functional role in the modulation of CB1 stimulation.

**Figure 2. GCPR activation and [35S]GTPS binding assay mechanisms.** a) Ligand binding to the receptor leads to the exchange of GDP for GTP on the subunit of the GCPR; downstream effects are activated by the G-GTP and G subunits. GTPase hydrolyses the GTP in the G-GTP subunit to GDP, thus turning off the receptor and allowing the subunits, G and G to reassociate. b) [35S]GTPS is substituted for GTP in the reaction. [35S]GTPS exchanged with GDP, activating the receptor, however [35S]GTPS cannot be hydrolyzed by GTPase, consequently allowing for the G-bound [35S]GTPS to accumulate during the reaction period. (Adapted from Ref 8.)

The criteria which triggers CRIP1a to modulate CB1 stimulus could conceivably be completely dependent upon the efficacy of the ligand stimulating CB1, as Smith et al. (2015) observed CRIP1a modulation of CB1 occurring for the three full agonist and the inverse agonist, while not the partial agonists7. However, as full agonists and inverse agonists entice more extreme stimulation outcomes of CB1 receptors, CRIP1a modulation could conceivably be triggered when CB1 stimulation exceeds a particular range. The purpose of the experiment in this proposal is to test whether ligand type or stimulus beyond a range is the trigger for CRIP1a modulation of CB1.

**II. Experiment**

The aim of this experiment is to determine if CRIP1a modulates CB1 stimulation in the presence of inverse agonists by the ligands’ intrinsic efficacies or has a range in which it does not affect CB1 stimulation with the use of [35S]GTPS binding assay. If CRIP1a modulation is dependent upon the ligands intrinsic efficacies, then CRIP1a would reduce the inverse agonism of the ligands. However if CRIP1a modulates based upon a range of CB1 stimulation, then CRIP1a would have less effect on CB1 stimulation in the presence of the less potent ligands.

II.A. [35S]GTPS binding assay

To assess CB1 stimulation, CB1 receptor activation while in the presence of the ligands would need to be quantified. The [35S]GTPS binding assay accomplishes this task by preventing deactivation of the receptor. Figure 2Ashows the mechanism of GCPR activation and Figure 2Bshows the mechanism when [35S]GTPS is introduced, effectively interfering with deactivation of the receptor by preventing hydrolysis of the radioactive [35S]GTPS8. The quantity of CB1 bound with [35S]GTPS is assessed by measuring the radioactivity with a scintillation counter.

II.B. [35S]GTPS experiment

**Figure 3. Structures of CB1 inverse agonists used.** The structures, from left to right are SR141716A , AM251, and AM281. AM251 and AM281 are structural analogs of SR141716A and are three and eight times less potent, respectively, than SR141716A. (Adapted from Ref 9.)

In order to assess CRIP1a modulation of CB1, Human Embryonic Kidney (HEK) cells first need to be transfected to stably express either CB1 (hCB1-HEK) or CB1 and CRIP1a (hCB1-HEK-CRIP1a). Receptor density for these cell lines would be assessed by [3H]SR141716A binding assay to ensure both cell lines have a similar receptor density. Basal binding, which is the constitutive activity of a receptor without ligands present, would need to be assessed for both cell lines with [35S]GTPS binding assay for calculation of percent stimulation of CB1 when in the presence of the ligands7.

The main experiment is [35S]GTPS binding assay performed on the CB1 receptor in both of the cell lines while in the presence of the ligands. The three ligands used are SR141716A, AM251, and AM281, which are all inverse agonist and therefore reduce constitutive activity of the receptor. These ligands were selected because AM251 and AM281 are both structural analogs of SR141716A, which was the first CB1 selective inverse agonist developed, and are three and eight times less potent, respectively, than SR141716A. Figure 3 shows the structural differences of the three ligands9. The ligands would be introduced to both hCB1-HEK and hCB1-HEK-CRIP1a cell lines and allowed to interact for a period of time, at which point the reaction would be terminated and radioactivity from [35S]GTPS bound to CB1 would be measured. To compare the stimulation of CB1 caused by the ligands, percent stimulation is calculated by (net stimulation of the receptor / basal binding) X 100%. Net stimulation is calculated by ligand stimulation – basal binding7.

**III. Discussion**

If CRIP1a modulation of CB1 stimulation is triggered based upon the intrinsic efficacy of the ligand, the receptor in hCB1-HEK-CRIP1a cells will be stimulated more than the receptor in hCB1-HEK cells, as Smith et al. (2015) observed CRIP1a reducing the inverse agonism caused by SR141716A7. If CRIP1a modulation of CB1 stimulation is triggered when CB1 stimulation expands past a range, then CRIP1a’s effect on stimulation levels would be less pronounced with the less potent ligand. CRIP1a would be expected to reduce inverse agonism of SR141716A the most, followed by AM251, and AM281 inverse agonism would be reduced the least.

Current research has observed CRIP1a playing a regulatory role upon CB1. The determination of what triggers CRIP1a to modulate CB1 stimulation is a necessary component to expanding the knowledge of CRIP1a’s regulatory nature. With the limited knowledge that has been gathered to date, there is much that is unknown about CRIP1a and much that needs to be understood. If CRIP1a’s modulation is triggered by exceeding a range of CB1 stimulation, the next step is to find the exact range of stimulation required to trigger CRIP1a to modulate CB1. Future experimentation would be beneficial in uncovering the interactions between CRIP1a and CB1 receptors, as this would continue to expand the understanding of the endocannabinoid system as a whole.

**IV. References**

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