Pain relief of cannabinoids due to K_{ATP} pathway and its connections to the opioid pathway in mice

Lauren Messerschmidt, Amanda Klein
Department of Pharmacy Practice and Pharmaceutical Sciences, University of Minnesota, Duluth MN

Introduction

Opioids are known to be addictive drugs, and long-term use may lead to overdose or death. To combat the opioid epidemic, different therapies should be developed and used for pain management. One potential alternative to prescription opioids is cannabis. The science of cannabinoids, the chemical compounds in cannabis, is relatively new research.

There are two primary cannabinoid receptors: CB1 and CB2, both being G-protein coupled receptors (Pertwee, 2006). The receptors are especially sensitive to THC and cannabidiol, both components in cannabis, which are thought to aid in pain relief. The hypothesized pathway begins with a cannabinoid binding to its receptor, which inhibits adenylyl cyclase. Adenylyl cyclase is responsible for producing cyclic adenylyl phosphate (cAMP) from ATP. Inhibiting adenylyl cyclase decreases the concentration of cAMP, resulting in the opening of ATP-sensitive potassium channel opening (K_{ATP} channels, Pertwee, 2006). This aligns with an accepted pathway for opioid signal transduction (Figure 2), and it is possible cannabinoids and opioids affect the same downstream potassium channels.

In this research, experimentation of mice was used to observe the potassium channel connections between the opioid and cannabinoid pathways. This was the starting point in finding a potentially safer therapy than opioids but with similar pain relief efficacy.

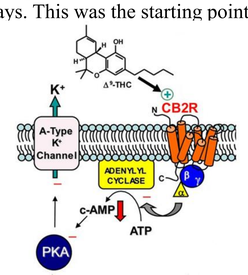

Figure 1. The proposed cannabinoid pathway. When the THC-component binds to the CB receptor, adenylyl cyclase is inhibited, decreasing the concentration of cyclic-AMP. Ultimately, this opens the potassium channel. When adenylyl cyclase is activated, the concentration of cyclic-AMP increases and the potassium channel is inhibited (Dhopeshwarkar et al. 2014).

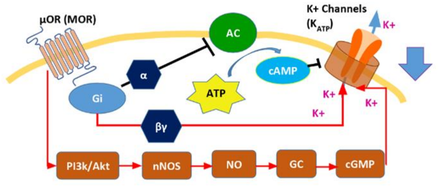

Figure 2. The proposed and accepted morphine pathway. When the inhibitory G-coupled protein receptor binds morphine, adenylyl cyclase is inhibited, decreasing the formation of cyclic-AMP. This opens the potassium channels, allowing an influx of potassium out of the cell (Cunha et al. 2010).

Methods

Part I: Mechanical Paw Thresholds

Withdrawal latencies were assessed at baseline and over a duration of sixty minutes after injection. The mice were injected subcutaneously in the loose skin on the neck (i.e. subcutaneous injection) with one hundred microliters of WIN 55212-2 (5 mg/kg, CB1 and CB2 agonist), GW 405833 (30 mg/kg, CB2 agonist), or vehicle (5% dimethylsulfoxide (DMSO) in saline (0.9% NaCl)). Paw withdrawal thresholds were measured on the left and right hind paws with a handheld electronic Von Frey hair (2392, IITC Life Sciences, Woodland Hills, California) on a wire mesh floor. Mice were grouped according to sex and genotype, with each group consisting of two to five mice. The knockout mice lacked the SUR1-subtype of ATP-sensitive potassium channels (K_{ATP} channels), wild-type mice had fully functional K_{ATP} channels, and heterozygous mice were an intermediate of the SUR1 wild-type and knockout mice.

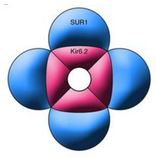

Figure 3. Protein octamer of the potassium channel. The outer tetramer, SUR1, was missing in the knockout mice (Ashcroft et al. 2005).

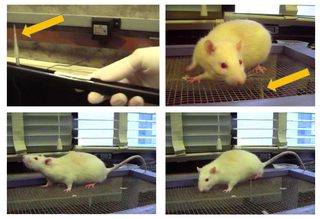

Figure 4. The mechanism of mechanical paw threshold. A handheld electronic Von Frey hair was used to measure pain response in hind paws of mice..

Part II: Quantitative PCR

Quantitative PCR was carried out for the genes Cnr1, Cnr2v1, and Cnr2v2. cDNA expression of each gene was measured in four mouse samples of sciatic nerve, dorsal root ganglia, and spinal cord, which was compared to the expression of cDNA in the same tissues in morphine tolerant mouse samples.

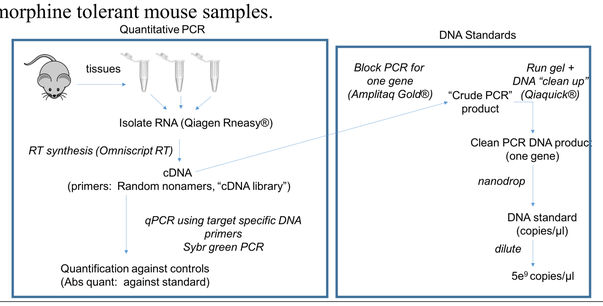

Figure 5. qPCR procedure. The tissues used were the spinal cord, dorsal root ganglia, and sciatic nerve. Primers for Cnr1, Cnr2v1, and Cnr2v2 genes were designed using sequences from NCBI primer design (https://www.ncbi.nlm.nih.gov/tools/primer-blast/).

Results

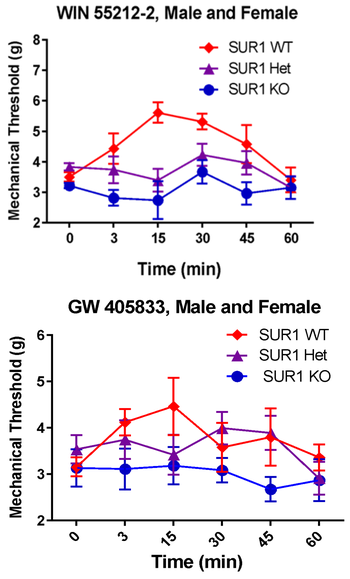

Figure 6. Mechanical threshold results after injection of WIN 55212-2 (CB1 and CB2 agonist) and GW 405833 (CB2 agonist) compounds. After injection of one hundred microliters of the compound at time=0, male and female SUR1 mice in wild-type (WT), heterozygous (Het), and knockout (KO) genotype groupings were measured with mechanical paw threshold every fifteen minutes for sixty minutes.

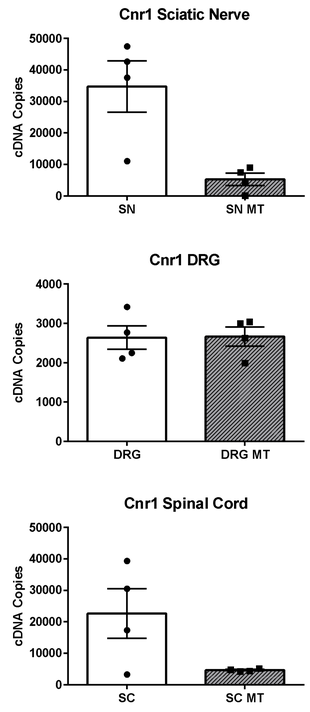

Figure 7. Results of qPCR for the Cnr1 gene. cDNA was synthesized from RNA samples in sciatic nerve (SN), dorsal root ganglia (DRG), and spinal cord (SC) of mice and compared to expression in the same tissues in morphine tolerant (MT) mice. Four samples were used for each tissue.

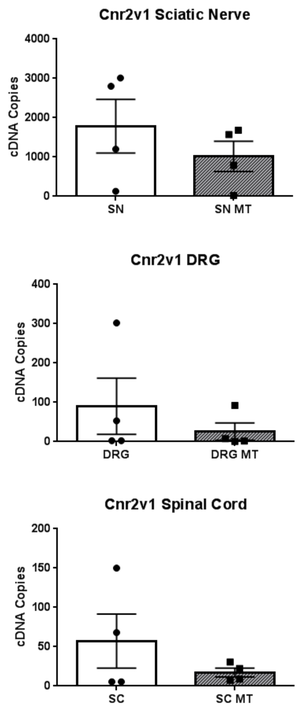

Figure 8. Results of qPCR for the Cnr2v1 gene. cDNA was synthesized from RNA samples in sciatic nerve (SN), dorsal root ganglia (DRG), and spinal cord (SC) of mice and compared to expression in the same tissues in morphine tolerant (MT) mice. Four samples were used for each tissue.

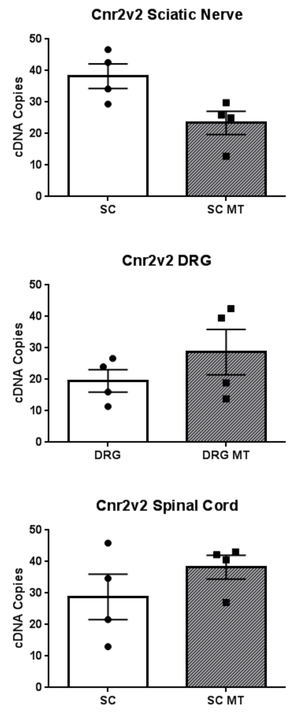

Figure 9. Results of qPCR for the Cnr2v2 gene. cDNA was synthesized from RNA samples in sciatic nerve (SN), dorsal root ganglia (DRG), and spinal cord (SC) of mice and compared to expression in the same tissues in morphine tolerant (MT) mice. Four samples were used for each tissue.

WIN 55212-2 injections increased mechanical threshold for wild-type and heterozygous mice, and there was a significant difference between wild-type, heterozygous, and knockout mice over time (repeated measures ANOVA, $F(10, 100) = 2.312$, $p = 0.0172$) (Figure 6). GW 405833 injections also increased the mechanical threshold for wild-type and heterozygous mice, and there was a significant difference comparing wild-type, heterozygous, and knockout mice, but not over time (repeated measures ANOVA, $F(2,19) = 4.614$, $p = 0.0233$) (Figure 6).

The results of qPCR showed gene expression was typically lower in morphine tolerant tissues compared to the control tissues (Figure 7, 8, 9), with significant differences in Cnr1 and Cnr2v2 sciatic nerve tissues (unpaired t-test, $p = 0.0125$ and $p = 0.0328$, respectively). Cnr2v2 had very low expression in all three tissues (Figure 9), while Cnr1 showed very high expression in the tissues (Figure 7). Cnr2v1 had high expression in the sciatic nerve but low expression in the dorsal root ganglia and the spinal cord (Figure 8). Cnr2v2 had higher expression in morphine tolerant dorsal root ganglia and spinal cord (Figure 9), but Cnr1 and Cnr2v1 had overall lower expression in the morphine tolerant tissues (Figure 7, 8).

Summary

The results of the mechanical paw threshold experiment support the hypothesis that cannabinoids can open at least one of the same potassium channels as morphine. Both the wildtype and heterozygous SUR1 mice, possessing functional K_{ATP} channels, experienced an increase in mechanical threshold after administration of the CB1 and CB2 agonist compounds. An increase in mechanical threshold correlates with an increased pain tolerance in the mice. In the knockout mice, lacking the SUR1 subtype of the K_{ATP} channel, there was no change in mechanical threshold.

Quantitative PCR supported the presence of CB1 and CB2 receptors in the pain pathway, specifically in the sciatic nerve, dorsal root ganglia, and spinal cord of mice. The Cnr1 gene, coding for the CB1 receptor, was highly expressed in all three tissues, more so in the sciatic nerve and spinal cord than the dorsal root ganglia. Cnr2v1 and Cnr2v2 are variants of genes that code for the CB2 receptor, and they had significantly lower expression in the tissues as compared to Cnr1. This supports CB1 as the primary receptor responsible for pain relief with CB2 as a less essential receptor. Compared to morphine-tolerant tissues, the control tissues had typically more expression of the genes, seen in the results for Cnr1 and Cnr2v1. This could be attributed to the down regulation of receptors as a safety mechanism.

Conclusion

This research was merely a starting point in determining the pain efficacy of cannabinoids. The data supported the hypothesis of cannabinoids opening potassium channels to provide pain relief, similar to the mechanism of morphine, but the intermediates of the proposed pathway are yet to be confirmed. Further experimentation will focus on determining the cannabinoid pathway in comparison to the opioid pathway to explore a potential solution to the opioid epidemic.