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Differential Modulation Of Oxycodone Reward And Analgesia By A Cannabinoid Derivative

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DIFFERENTIAL MODULATION OF OXYCODONE REWARD AND ANALGESIA BY A CANNABINOID DERIVATIVE

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ABSTRACT

This study sought to determine whether a cannabidiol derivative, CBD-val-HS, could attenuate the development of oxycodone reward while retaining its analgesic effects. In Experiment 1) animals were enrolled in the conditioned place preference paradigm and received either saline or oxycodone in combination with one of four doses of CBD-val-HS using 3 sets of drug-/no drug-conditioning trials. Experiment 2) sought to determine whether a dose of CBD-val-HS that blocks opioid reward administered alone or in combination with a sub-analgesic or analgesic doses of oxycodone would affect nociceptive processes in the hotplate and abdominal writhing assays. Results from this study demonstrated CBD-val-HS can attenuate the rewarding effects of oxycodone place preference at 8.0 mg/kg and it is void of rewarding or aversive properties. Further, CBD-val-HS alone produced analgesic effects in both nociceptive assays but was most effective when compared to oxycodone against thermal nociception. Interestingly, there was a differential interaction of CBD-val-HS+oxycodone across the two nociceptive assays producing subadditive responses on the hotplate assay while additive responses were observed in the abdominal writhing assay. These findings suggest CBD-val-HS, a non-addicting analgesic compound, could prove useful in pain management and addiction treatment settings.

DEDICATION

I would like to dedicate my dissertation to my grandmother, Dorthy Smith Harris and my parents to whom I owe all of my achievements. I would also like to dedicate this in memory of my grandparents, Merle Ann Lusich Stoufflet, Aubin G. “Fritz” Harris, Sr., and David Matthew Stoufflet,

“There is so much good in the worst of us, bad in the best of us, behooves any of us to judge the rest of us judge the rest of us”…

Ida Ruth Cranford Smith

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INTRODUCTION

PAIN

Pain is a biological response to noxious stimuli in our environment that initiates escape responses from painful stimuli and is critical for survival. Pain is defined as "an unpleasant sensory and emotional experience associated with actual or potential tissue damage" ("IASP Taxonomy," 2016). Over 100 million Americans suffer from pain daily accounting for 80% of physician visits (Voscopaoulos & Lema, 2010; Pain: Hope through research," 2016; Li & Zhang, 2012). The economic burden of pain in the United States is estimated to be $636 billion annually due to treatment, loss of productivity, and long-term disability (Li & Zhang, 2012; "Relieving pain in America," 2011; Nahin, 2015). Depending on the intensity and duration of a noxious stimulus (Voscopaoulos & Lema, 2010) pain can become insufferable, diminishing the quality of life.

Depending on the duration of healing, pain can be either acute or chronic. Acute pain is caused by sudden activation of pain nociceptors. Noxious stimuli in acute pain are identifiable and allow immediate removal of the stimulus oftentimes resolving within 3 weeks (Voscopaoulos & Lema, 2010; Barkin & Barkin 2001). Common types of acute pain include upper respiratory tract infections, headache, tooth pain, and post-operative surgical pain (Rice,

Smith, & Blyth, 2016; "Fact sheets," 2016). In chronic pain, nociceptor pathways remain active after a noxious stimulus is removed and persists after a reasonable time for tissue to heal. Chronic pain can last for 12 weeks or longer (Benzon et al., 2011). According to the American Academy of pain, 1.5 billion individuals worldwide are affected by chronic pain with cancer pain, neuropathy, and arthritis the most common (Dale & Stacey, 2016). Pain is a multifaceted disorder with a variety of etiologies (Li & Zhang, 2012) and pharmacological treatments.

OPIOIDS

Pharmacotherapies for treating pain include non-steroidal anti-inflammatory (NSAIDs), antidepressants, anticonvulsants, and opioids. Opioids are highly efficacious and are considered the "Gold Standard" in pain treatment (Li & Zhang, 2012). The family of opioids can be divided into opioids and opiates. Opiates are alkaloids derived from the opium poppy plant and include Morphine, Codeine, Heroin, and Opium. Opioids are synthetic or partly synthetic drugs that mimic the actions of opiates. These include hydrocodone, fentanyl, and oxycodone. Opioids show high efficacy in treating a wide range of pain related injuries and diseases and are the most prescribed treatment for chronic pain.

Opioids produce analgesia through binding to opioid receptors located throughout the peripheral and central nervous system (CNS). Mu and kappa receptors are located in the periaqueductal gray (PAG) while delta receptors in structures of the forebrain and hindbrain (Toll et al., 2015). The PAG projects to limbic targets upstream to modulate emotional pain, and downstream to the nucleus raphe magnus, and terminate on pain inhibitory neurons in the dorsal horn of the spinal cord (Ossipov, Dussor, & Porreca, 2010).

Opioid binding to the PAG enhances descending inhibition and releases neurotransmitters (NT) that stimulate inhibitory interneurons in the dorsal horn. Binding leads to inhibition of the

afferent pain fiber and blocking the transmission of pain neurotransmitters calcitonin gene-related peptides (cGRP), glutamate, and Substance P (Sub P) and ultimately block pain impulses and produce analgesia (Williams, 2008; Ossipov, Dussor, & Porreca, 2010). Although highly efficacious in treating pain, opioids are not without shortcomings. Because opioid receptors are abundantly found in the PNS and CNS, they can produce a number of side effects that limit their therapeutic use.

Side effects

Approximately 80% of patients treated with opioids suffer from adverse side effects (Kalso, 2004) that diminish quality of life. Further, drugs with selectivity to specific opioid receptors produce their own set of side effects. Most opioid analgesics are mu receptor agonists and produce side effects of sedation, vomiting, respiratory depression, nausea, and sleep disturbances as well as constipation. The magnitude of these side effects often depends on short or long term use (Cepeda, 2003; Benyamin et al., 2008).

The greatest concern when prescribing opioids is respiratory depression and this side effect is common in acute opioid use (Dahan, Aarts, & Smith, 2010). Opioids affect respiratory centers that receive peripheral inputs from chemoreceptors responsible for detecting levels of oxygen and carbon dioxide, as well as stretch receptors that respond to lung inflation (Mitchel, 1980). Opioids can dose dependently produce inhibition of chemoreceptors by binding to mu and delta receptors. This inhibition decreases responsiveness to carbon dioxide levels resulting in depression of breathing that can be fatal (White & Irvine, 1999).

Opioid-induced nausea and vomiting is reported in 25 to 40% of patients (Swegle & Logemann, 2006; Meuser et al., 2001). Patients report this as the most distressing side effect of opioid use (McNicol et al., 2003). Mu opioid receptors are abundant in the area postrema (Smith

& Laufer, 2014), a brainstem region responsible for detecting toxins in the bloodstream and triggering the vomit reflex. Even low doses of opioid analgesics activate mu receptors leading to nausea and vomiting.

Sedation and sleep disturbances are another consequence of opioid use. Opioid-induced sedation is thought to be mediated by anticholinergic effects that can be improved through opioid rotation and or reduction as well as with the addition of a psychostimulant (McNicol et al., 2003). Although the mechanism is unknown, opioids interfere with these NTs responsible in mediating sleep cycles. Altering these NTs decreases the amount of time REM and restorative sleep and further effects arousal during wakefulness (Slatkin & Rhiner, 2004).

Outside the CNS, opioid receptors can be found in the gastrointestinal (GI) tract. Opioids binding to mu receptors in the GI tract decreases bowel motility and peristalsis, that lead to constipation (McNicol et al., 2003; Benyamin et al., 2008). This is the most common side effect of mu opioid agonists with 40% -95% of patient's report they suffer from constipation (Kalso, 2004). This side effect does not improve over time (Shug et al., 2003) and can occur with a single dose of morphine (Swegle & Lagemann, 2006). Patients suffering from constipation often develop hemorrhoids, bowel obstruction, and potential bowel rupture (Kurz & Sessler, 2003).

While opioids remain the mainstay in pain management settings due to their full efficacy across a range of chronic pain syndromes, their side effect profile limits quality of life. Perhaps the most disconcerting side effect of opioids use is their ability to affect reward pathways leading to the development of addiction.

Reward Pathway

The rewarding effects of opioids have been extensively researched using the condition place paradigm (CPP) and rodent models of self-administration (SA). CPP is based on principles

of associative learning whereby animals prefer or avoid environments previously paired with reinforcing or aversive drugs, respectively. SA is based on operant conditioning whereby animals elicit responses, such as a lever press, to receive a drug. Rewarding drugs will increase behavioral responses reflective of drug seeking and taking behavior. Rewarding effects of opiates have been largely attributed to binding with mu receptors. Agonists on mu opioid receptor have shown to increase opiate self-administration (O'Connor, Chapman, Butler, & Mead, 2010) while blocking this receptor attenuates self-administration (Weeks & Collins, 1976, Koob et al., 1984). This effect has also been demonstrated in CPP where mu receptor agonists are well known to produce place preference (Tzschentke, 1998) while mu receptor knockout mice do not develop opioid place preference (Matthes et al., 1996).

The rewarding effects of mu agonists are related to structures within the mesocorticolimbic system that is dense with opioid receptors (Mansour, Fox, Burke, Akil, & Watson, 1995). This "reward pathway" is composed of dopaminergic neurons originating in the ventral tegmental area (VTA) that projects to the Nucleus accumbens (NAc) and the ventral pallidum and are responsible for GABA release. Additional structures involved in this pathway are the prefrontal cortex (PFC), amygdala, and the mediodorsal thalamus and are responsible for glutamate release (Mansour, Fox, Burke, Akil, & Watson, 1995). The two most researched structures believed to be the primary source of these reinforcing effects are the VTA and the NAc. (Le Merrer, Becker, Befort, & Kieffer, 2009).

The VTA and the NAc are important in relaying information about rewarding or aversive stimuli in the environment and motivating behavior associated with reward. Increase dopamine (DA) in these areas is associated with reinforcing and rewarding values of drugs of abuse (Spanagel & Weiss, 1999; Wise & Rompre, 1989). Indeed, opioids indirectly increase DA levels

through activation of presynaptic GABA neurons that in turn inhibit GABA release in the VTA. GABA inhibition allows DA accumulation in the NAc causing the pleasurable effects (Johnson & North, 1992; (Ting-A-Kee & van der Kooy, 2012). These pleasurable effects have been demonstrated in a multitude of animal models. For example, blocking opioid receptors in the NAc has shown to decrease self- administration (Vaccarino, Bloom, & Koob, 1985). Lesions to both the VTA and the NAc blocks morphine SA (Smith, Guerin, Co, Barr, & Lane, 1985) and conditioned place preference (Bals-Kubik, Ableitner, & Shippenberg, 1993). Repeated exposure to opioids can lead to long term neuroadaptations of mesolimbic DA neurons that underlie addiction (Van Bockstaele, Reyes, & Valentino, 2010).

DSM criterion for diagnosing addiction includes the development of tolerance, withdrawal, and occurs when "The substance is often taken in larger amounts and over a longer period than was intended" (American Psychiatric Association, 2013). Addiction is a result to prolonged drug exposure that causes neuroadaptations in the mesolimbic pathway, striatum, prefrontal cortex, hippocampus, and the amygdala. Repeated drug use produces alterations in signal transduction as well as decreased opioid receptor sensitivity resulting in tolerance (Dumas & Pollack, 2008). Tolerance is a markedly diminished drug effect whereby higher doses are required to achieve the initial drug response (Dumas & Pollack, 2008). Opioids inhibit the release of norepinephrine in the locus coeruleus. After prolong opioid use, adrenergic receptors are upregulated to account for the excess binding of opioids. Upon opioid cessation, the locus coeruleus releases an overabundance of norepinephrine precipitating withdrawal symptoms that include high heart rate, increase blood pressure, runny nose, tearing of the eyes, diarrhea, and constipation (Ballantyne & LaForge, 2007). Because endogenous opioids are unable to maintain

equilibrium, the body becomes physically dependent to opioids. To prevent withdrawal symptoms opioid use is reinstated.

HISTORY OF OPOIOD USE

Opioid use dates back to 3400 BC when the Sumerians first cultivated the opium poppy referred to as Hul Gil or "joy plant" due to the euphoria it produced. However, the first record of opium poppy use to relieve pain was by Egyptians and its use spread to many other civilizations. By 1170, opium had reached western medicine and was often used in surgeries where opium soaked rags were placed over the nose of patients undergoing surgery (Wilkerson, Kim, Windsor, & Mareiniss, 2016).

By the 19th century, opium became a key ingredient in western medicine for pain relief, sleep aid, and even to keep children quiet (Iverson et al., 2009). In 1805, the "inducing-factor" morphine was extracted from opium by German researcher Friedrich Livenstein (Rosemblaum et al., 2008). The industrial manufacturing of morphine followed soon thereafter but it was not until the invention of the hypodermic needle that morphine use became widespread. Subcutaneous administration of morphine allowed rapid delivery of drug and was thought to lack side effects produced by oral administration (Sabatowski, Schafer, Kasper, Brunsch, & Radbruch, 2004). Morphine use quickly rose to use during the civil war leaving many soldiers addicted at the war's end. This "soldier's disease" brought light to the addictive qualities of morphine and led to the research of less addicting analgesics.

The next shift in opioid use occurred following the synthesis of Diacetylymorphine in 1874 by Charles Adler Wright. Diacetylymorphine was shown to have cough suppressant properties in animal models (Sabatowski, Schafer, Kasper, Brunsch, & Radbruch, 2004). Around this time, pneumonia and tuberculosis was the leading cause of death popularizing

medicating with heroin. In 1898, the pharmaceutical Bayer released Diacetlymorphine registered under the name of heroin. Heroin was marketed as an effective pain reliever that was less addictive than morphine making heroin a "wonder drug". By 1899, Bayer was producing one ton of heroin a year and exporting it to 23 countries. Furthering its use, the American Medical Association approved heroin as a safer substitution for morphine. During this time, physicians noticed the addictive qualities of heroin. Without regulation, heroin use spread fast as users learned euphoric effects could be achieved when injected. By the early 1900s, an estimated 300,000 people suffered from addiction with many being civil war veterans (Levinthal, 1985). During this time, addicts would collect scrap metal to sustain their habit coining the term "junkie" (Daly, 2014). Due to the rise of addiction, Bayer pulled its Diacetylmorphine off the market in 1911 (Courtwright, 1992).

In response to the high rates of addiction, President Theodore Roosevelt's administration set out to end to the opioid addiction crisis in the United States. At this time, the United States consumed more "habit-forming drugs per capita" but with fewer safeguards (Marshall, 1911). The Harrison Act of 1914 became the United States' first drug law criminalizing the non-medical use of opium. This act made it illegal to prescribe narcotics to those who were addicted ("Harrison Narcotics tax act, 1914 - full text," 1914). This act brought criminal charges to tens of thousands physicians resulting in imprisonment for many (Daly, 2014). Physicians that were able avoid prison sentences were left with tarnished medical careers. With fear of imprisonment, many physicians avoided treating patients with opioids resulting in the under treatment of pain for the next 60 years.

The view of opioids in pain management shifted beginning in 1995 with a joint statement released from the American Pain Society and the American Academy of Pain Management

arguing physicians were under treating pain (Wilerson et al., 2016). Both groups claimed less than 1% of patients in pain management formed opioid addiction. This data point was taken from a study published in 1980 in The New England Journal of Medicine stating "the development of addition is rare in medical patients with no history of addiction" (Rosenblum, Marsch, Joseph, & Portenoy, 2008). These groups lobbied that pain should be recognized as the "fifth vital sign" and advocated doctors to increase opioid prescriptions ("Assessment of pain," 2006). Purdue Pharma, who helped fund the American Pain Society and the American Academy of Pain, released OxyContin in 1996 marking the beginning of the current opioid epidemic.

Opioid Epidemic

Oxycodone is a semisynthetic opioid that binds to mu opioid receptors providing relief for over 12 hours ("Report to congressional requesters," 2003). In contrast to other opioids, oxycodone provides the benefits of pain relief in fewer dosages and allows patients uninterrupted sleep. These benefits made oxycodone a highly desirable opioid in pain management. Indeed, in its first year on the market OxyContin sales reached $45 million. In 2010, sales exceeded $3.1 billion and accounted for 30% of opioid analgesics on the market ("Oxycontin Abuse and diversion and Efforts to Address the Problem," 2013).

Although beneficial in treating pain, the dangers of oxycodone can be dated to 1960 when it was classified in The Dangerous Drugs (Amendment) Ordinance in 1960 by the United Nations ("Oxycodone," n.d.). It was not until its release in 1996 that the abuse liability would be the forefront of concern. OxyContin was first marketed to physicians as a safe non-addicting opioid (Zee, 2009). Four years after its release, however, OxyContin was the leading drug of abuse in the United (Cierco, Inciardi, & Munoz, 2005).

The peak of the opioid epidemic was in 2010 when opioids were responsible for more than twice as many fatalities than both heroin and cocaine (Center for Disease Control and Prevention, 2013). In 2015 alone, prescription opioids accounted for approximately 22,000 overdose deaths (CDC, 2016) which is the equivalent of 42 deaths per day (Rudd, Seth, David, & Scholl, 2016). In 2012, it was estimated that up to 36 million people world-wide abused opioids and 2 million American are dependent on prescription opioids (Substance Abuse, 2014). Opioid related emergency room visits from 2004-2011 have increased by 183% (Wilkerson, Kim, Windosr, & Mareiniss, 2016) with OxyContin accounting for 175,949 emergency room visits in 2009 alone ("Drug-related hospital emergency room visits," 2011). The economic burden opioid abuse in terms of loss of productivity and drug abuse treatment costs $53 to $72 billion annually (Hanse, Oster, & Edelsberg, 2011).

Despite this global epidemic, physicians continue to prescribe opioids at an alarming rate. With the United States constituting around 5% of the world's population it consumes 80% of the global opioid supply of oxycodone (Manchikanti & Singh, 2008). According to the CDC, "Providers wrote nearly a quarter of a billion opioid prescriptions in 2013—with wide variation across states. This is enough for every American adult to have their own bottle of pills" (CDC, 2016).

TREATING OPIOID ADDICTION

Pharmacological treatment for opioid addiction involves either opioid replacement therapy (ORT) or detoxification (Stotts et al., 2010). ORT involves replacing an illegal opioid with an opioid that produces a weaker euphoric effect. The ultimate goal in ORT is to decreasing drug seeking behavior in order to stabilize patients and enroll them into behavioral therapies. Detoxification therapy is a medically controlled withdrawal from a drug allowing clearance of

opioids from the patient. During detox therapy, patients experience withdrawal symptoms of agitation, hot and cold flashes, nausea and vomiting that can last from hours to days (Kelber, 2007). There are three main classes of pharmacological treatments used in ORT and detoxification therapy. These classes include opioid agonists, opioid antagonists, and non-opioid medications.

Opioid agonists bind to opioid receptors and mimic the effects of endogenous opiates (Julien, 1998). These agonists replace opioids of abuse and are utilized in both opioid maintenance and detoxification (Stotts et al., 2010). Most drugs of abuse produce a cycle of an intense short term euphoria followed by an intense "low" or crash that leads to craving initiating drug seeking behavior. ORT agonists produce weaker and long lasting euphoria. The goal in ORT is to reduce withdrawal symptoms, drug seeking behavior, and eventually taper individuals off agonists to reach full abstinence. Further, these medications block or decrease the euphoric effects of subsequent heroin or opioid use acting as competitive antagonists. The three main agonists used in treating opioid addiction and dependence are methadone, levomethadylacetate (LAAM), and buprenorphine.

Methadone and LAAM are full opioid agonists that bind to mu opioid receptors. Methadone has a short half-life of 22 hours requiring daily administration while the half-life for LAAM is 4 days (Strain & Stitzner, 2006) and requires administration thrice weekly (Ling & Compton, 2005; Stotts et al., 2010). Both methadone and LAAM are effective in treating opioid dependence (Johnson et al., 2000) and addiction (Longshore, Annon, Anglin, & Rawson, 2005) and LAAM more effective than methadone in reducing heroin use (Clark et al., 2002). However, many patients treated with LAAM switch to methadone due to its adverse side effects and risk of

cardiac ventricular arrhythmia (Wieneke et al., 2009; Clark et al., 2002) making methadone the first line of treatment for opioid addiction (Veilleux et al., 2010).

While methadone and LAAM produce weak euphoria, users can become addicted to these compounds (Veilleux et al., 2010). Many consider this as "replacing one addiction with another" ("Methadone abuse," 2013). In efforts to cut down on abuse and diversion, methadone and LAAM are classified as Schedule II drugs and are given in controlled environments (methadone maintenance programs). Even with tight regulation, methadone abuse is highly prevalent. A 2012 study reported 2.5 million people over the age of 12 reported that they abused methadone at one point in their lifetime ("Methadone abuse," 2013). In 2011, methadone accounted for 26% of total deaths from opioid overdoses in the United States alone (National Center for Health Statistics, 2014).

Unlike methadone and LAAM, Buprenorphine is a partial agonist on mu opioid receptors and an antagonist at kappa opioid receptors. This buprenorphine binding profile is associated with fewer adverse side effects and a decrease risk of unintentional overdose compared to full opioid agonists (Stotts et al., 2010; Walsh, Preston, Bigelow, & Stitzer, 1995). Buprenorphine is classified as a Schedule III drug due to its low abuse liability and requires less monitoring than methadone. To further minimize its abuse liability, buprenorphine is often combined with naloxone which produces antagonist effects when opioids are abused (Orman & Keating, 2009; Whelan & Remski, 2012). Another advantage of buprenorphine is its moderate withdrawal symptoms following prolong use in comparison to morphine, fentanyl, and methadone (Tzschentke, 2002; Walsh & Eissenberg, 2003). Buprenorphine's long lasting effects have been shown to block the effects of 120 mg dose of morphine for up to 29.5 hours (Jasinski et al., 1978). Buprenorphine's efficacy in treating opioid addiction has met mixed reviews. The

disadvantage of buprenorphine is its weak effects on mu receptors where high activity on mu receptors have shown higher efficacy in blocking the effects of opioids. Studies have also shown buprenorphine can produce euphoria in non-opioid dependent individuals with an abuse potential lower than a full opioid agonist (Baumevieille et al., 1997).

A second approach to treating opioid addiction is with opioid receptor antagonists that block opioid binding rendering them ineffective. These antagonists are commonly used to accelerate detoxification (Stotts et al., 2010). The main opioid antagonist in treating opioid addiction and dependence is naltrexone. Unfortunately, there is a low compliance rate using naltrexone; up to 80% of patients drop out of naltrexone treatment within the first six months (Coviello, Cornish, Lynch, Alterman, & O'Brien, 2010). Further, studies have shown naltrexone's efficacy is no better than placebo, buprenorphine, or addicts who do not receive medication (Bart, 2012; Minozzi et al., 2011).

Non-opioid based pharmacotherapies represents a third strategy used in opioid detoxification and can reduce the intensity of withdrawal symptoms. Opioids inhibit the release of norepinephrine in the locus coeruleus. Following prolong opioid use adrenergic receptors are upregulated to account for the excess binding of opioids. Upon discontinuing opioid use, the locus coeruleus releases an overabundance of norepinephrine precipitating symptoms of high heart rate, increase blood pressure, runny nose, tearing of the eyes, diarrhea, and constipation. Alpha-2-adrenergic agonists such as clonidine and lofexidine are commonly used to mitigate withdrawal symptoms through their binding in the locus coeruleus. Both decrease release of norepinephrine and reduce withdrawal symptoms. Clonidine has been associated with severe hypotension making lofexidine the better choice in opioid detoxification therapy.

Prevention of Opioid Addiction

Much of today's opioid crisis is attributed to the use and misuse of opioid analgesics in pain settings. Pharmaceutical companies are currently working on abuse-deterrent formulations (ADF) of opioids for pain management.

The first abuse-deterrent strategy was the introduction of tamper resistant opioid formulations. Tamper resistant formulations create barriers on pills that physically and chemically prevent crushing or dissolving opioids. For example, Remoxy is slow release formulation of oxycodone in tamper proof tablets that is difficult to crush or dissolve. However, this strategy has been faced with mixed success in decreasing drug abuse. In 2008, King Pharmaceuticals submitted Remoxy for FDA approval but their application was rejected due to little data supporting its ability to reduce abuse (Moorman-Li et al., 2012). These reformulated compounds vary in analgesic efficacy and side effect profiles across patients that often lead physicians to switch or "tailor" opioid treatment following initial treatment. However, adequate pain management is a major challenge with only 3 tamper proof formulations approved by the FDA. Further, it is unknown if these tamper resistant formulations may affect opioids efficacy and tolerability (Pappagallo & Sokolowska, 2012).

A second strategy is the development of controlled-release opioid formulation. The concept is to produce a slow but steady release of opioids that minimizes abuse by avoiding a large surge in blood levels associated with euphoria. Purdue Pharma manufactured a controlled-release formulation of OxyContin and was marketed to curb abuse liability. Unfortunately, the FDA was not aware that this formulation could be crushed, dissolved in water, and injected producing rapid absorption and euphoria. This formulation consequently increased the abuse and misuse of OxyContin (Rappaport, 2008).

The uses of agonist-antagonist opioid combinations have also been introduced as a potential ADF. For example, Suboxone consists of the partial mu opioid agonist buprenorphine and the mu inverse antagonist naloxone. Naloxone has poor oral bioavailability and does not interfere with the analgesic properties of buprenorphine. When Suboxone is misused by injection, naloxone becomes active and rapidly blocks mu opioid receptors and precipitates withdrawal in opioid dependent patients (Moorman-Li et al., 2012; Katz, 2008). Although marketed as being effective in deterring abuse, currently no data are published data to support these claims (Katz, 2008).

Another ADF approach is the addition of aversive ingredients to opioids that produce unpleasant effects when misused. For example, adding capsaicin, a component of hot chili peppers, to opioids has been suggested. When consumed orally, capsaicin does not produce any aversive effects. However, if crushed, snorted, or injected, capsaicin produces intense burning. This strategy is rarely used due to the ethical controversy of using positive punishment as a means mean to deter opioid abuse (Katz, 2008).

The use of a pro-drug opioid formulation has been suggested as a potential abuse deterrent approach. A pro-drug is a biological entity that is inactive until it undergoes biotransformation in a rate-limiting step following ingestion. This class of compounds produces highly desirable pharmacodynamics effects that lead to gradual increases in and stable blood levels for long periods of active metabolites. There are several opioid pro-drug formulations developed but proof of concept studies has demonstrated the potential of such formulation to produce analgesic efficacy without an abuse liability.

A major effort in the pharmaceutical industry is in the development of analgesics that provide for full efficacy in pain management while preventing addiction. The clinical findings to

date indicate this effort has proven unsuccessful. However, a number of laboratories are engaged in pre-clinical research with hopes to develop opioid-based analgesic formulations that are void of an abuse liability but retain full analgesia across a broad spectrum of chronic pain conditions.

ENDOCANNABINOIDS AND OPIOIDS IN ANALGESIA AND ADDICTION

Cannabis sativa (marijuana) has been used for more than four centuries as an analgesic for a variety of pain conditions (Chiou et al., 2013). The two main constituents of cannabis are Δ^9-tetrahydrocannabinol (THC) and cannabidiol (CBD). These THC is presumed to exert analgesic effects by activation of cannabinoid 1 receptors (CB1R) in the CNS, and/or by activation of CB1R and CB2R receptors located on peripheral nerves (Chiou et al., 2013; Zogopoulos et al., 2008). THC, the primary psychoactive constituent in cannabis, binds to CB1R and CB2R affecting sites of nociception that process and encode harmful stimuli. CBD, the non- psychoactive constituent in cannabis, has a limited binding affinity to either CB1R or CB2R but is known to play a role in immune responses as well as nociception (Ameri, 1998; Nurmikko et al., 2007; Rahn and Hohmann, 2009; Chiou et al., 2013). When administered systemically, cannabinoids produce analgesic properties comparable to opioids in acute pain models (Chiou et al., 2013; Walker et al., 2001).

Studies have shown that opioid analgesia can be potentiated by cannabinoids producing supra-additive analgesic effects in a number of pain assays. For example, combinations of CBD or THC and morphine displayed synergistic effects in a murine abdominal writhing assay (Neelaktantan et al., 2014) and in arthritic models (Cox et al., 2007). THC has also shown to increase the antinociceptive properties of morphine in rodent tail-flick assays (Welch & Stevens, 1992). These effects are mediated by signaling interactions of CB1R and mu-opioid receptors co-

expressed in brain structures that modulate nociceptive responses (Wilson-Poe et al., 2008; Mansour et al., 1988) through a descending pain control circuit (Basbaum et al., 1984).

Cannabinoids, Opioids, and Reward

CB and opioid receptors are known to play a role in the reinforcing effects of drugs of abuse as demonstrated in a variety of rodent addiction models. The reinforcing effects of THC and opioids have been extensively researched in CPP. While THC and mu-agonists produce place preference, (Braida, Iosuè, Pegorini, & Sala, 2004; Lepore, Vorel, Lowinson, & Gardner, 1995; Tzschentke, 1998) antagonizing either receptor can influence these rewarding effects. For example, antagonizing CB1 receptors has been shown to block morphine place preference (Mas-Nieto et al., 2001). In addition, mu-opioid receptor KO mice receiving THC do not display place preference (Ghozland et al., 2002). These data suggest opioid and cannabinoid receptors interact to modulate rewarding effects of either drugs.

The activity of opioid and CB receptors in addiction has also been modeled in the rodent SA paradigm. Like drugs of abuse, CB1R agonists produce rewarding effects by increasing dopamine in the mesolimbic pathway producing pleasurable effects (Tanda, Pontieri, & Di Chiara, 1997). Animals exposed to THC show an increased self-administration of heroin (Solinas, Panlilio, & Goldberg, 2004) while antagonizing these CB1 receptors reduce heroin self-administration (Navarro et al., 2001). Further, morphine self-administration is reduced in CB1R knockout mice (Ledent et al., 1999). Taken together, CB1R agonists possess the ability to increase the abuse potential of opioids.

Unlike CB1Ragonists, CB2R agonists decrease dopamine in the ventral tegmental area and has shown to reduce the rewarding effects of drugs of abuse (Zhang et al., 2014). For example, agonizing CB2R reduces cocaine self-administration in mice (Xi et al., 2011; Zhang et

al., 2014). A similar effect on cocaine SA is seen in mice with overexpression of CB2R (Aracil-Frenandez et al., 2012). These data demonstrate CB2R agonists show potential in decreasing rewarding effects of opioids. Collectively these findings suggest reinforcing effects of drugs are mediated by localization of opioid and cannabinoid receptors. Further, CB1R agonists facilitate the reinforcing effects opioids while CB2R agonists mitigate these effects.

Recent research using an intracranial self-stimulation (ICSS) paradigm investigated CBD's effect on morphine reward. ICSS is an operant paradigm that allows rodents to self-administer rewarding electrical stimulation via electrodes implanted in the brain. Katsidoni and colleagues demonstrated CBD blocked the reward-facilitating effects of morphine on ICSS endpoints (Katsidoni, Anagnstou, & Panagis, 2012). The mechanisms that underlie these effects is unknown given CBD's low binding affinity to CB1Rs and CB2Rs. Nevertheless, the ability of CBD to decrease the rewarding effects of morphine in the ICSS model, this compound may have important abuse-deterrent properties in pain management settings.

It is unknown whether a CBD-opioid pharmacotherapy possesses qualities that prevent opioid abuse while retaining analgesic properties. The goal of this research is to determine whether CBD and a CBD derivative prevent opioid abuse while retaining analgesic properties. Such findings would lead to possible opioid formulations for use in pain management settings and mark the beginning of the end of prescription-initiated opioid addiction.

Ethical Considerations and IACUC Approval

All experiments were conducted in accordance with the American Psychological Association guidelines for the ethical treatment of nonhuman subjects and the policies of the University of Mississippi. All experiments described below have received approval by the University of Mississippi's Institutional Animal Care and Use Committee (IACUC) under protocol 15-022 on 18 May 2015.

CHAPTER II

PILOT STUDIES

Introduction

CBD has shown to mitigate morphine reward in a rodent model of intracranial self-stimulation (ICSS) (Katsidoni et al., 2013). Many paradigms model the development and maintenance of addition including intravenous (IV) self-administration and the condition place preference (CPP) paradigm. It is unknown if CBD actions on morphine reward can generalize to other models quantifying abuse liability. Therefore, this research sought to determine if CBD could attenuate morphine reward in a CPP paradigm.

Method

Subjects

C57BL/6 male mice (25-30 g) were group housed (n = 5) in a polycarbonate tub with soft bedding in a temperature and humidity controlled vivarium. Mice were maintained under a 12:12 hour light/dark cycle with lights on at 06:00. Food and water were available ad libitum. Mice acclimated to the vivarium colony room one week prior to behavioral testing. All experimental procedures were approved on 18 May 2015 by the Institutional Animal Care Committee at the University of Mississippi (Protocol # 15-022).

Apparatus

Five place preference chambers (Model MED-CPP-3013; Med Associates, St. Albans, VT) were used for these experiments. Each chamber has two stimulus-distinct conditioning

chambers (Black versus white colored walls and wire or mesh metal rod flooring; 16.75X12.70 cm) separated by a third central start chamber (7.25X12.70 cm; colored grey with a smooth solid floor). Guillotine doors permitted confinement/access to individual chambers.

Procedure

The groups in this study formed a 2x5 factorial design that combined 2 levels of morphine (saline and 2.5 mg/kg morphine) and 5 levels of CBD (vehicle and 4 doses). Morphine Sulfate (Research Biomedical International; Natick, MA) was dissolved in 0.9% saline to yield a dosage of 2.5 mg/ml. Cannabidiol (>98% purity) solutions of 2.5, 5.0, 10.0, and 20.0 mg/kg/mL (ELI Laboratories; Oxford, MS) were dissolved in a 5% ethanol/5% cremophor solution of injectable water. Mice received dual IP administrations of test compounds in a volume of 1 ml/kg.

Prior to behavioral testing, animals were allowed to acclimate to the testing room for at least 30 minutes. The CPP procedure consists of four phases: 1) a 15 min apparatus habituation trial, 2) a 15 min trial to establish baseline CPP scores, 3) six 45 min drug conditioning trials, and 4) a 15 min trial to establish post-conditioning CPP score. During the drug free habituation, baseline, and final preference trials animals were placed in the gray start chamber for a 5 minute adaption period. Following the adaption period, the guillotine doors were lifted allowing access to the entire apparatus. The test apparatus was thoroughly cleaned with 70% ethanol solution after each trial.

CPP scores were determined by $\frac{Time\ in\ Black}{Time\ in\ Black+White}$ and led to the establishment of the S+ chamber for drug conditioning whereby S+ assigned to the non-preferred compartment. From these CPP scores, baseline and post-conditioning scores were quantified as $\frac{Time\ in\ S+}{Time\ in\ S++\ S-}$.

Preference scores were calculated by taking subtracting post-conditioning and baseline CPP scores with positive values reflecting reward and negative values reflecting aversion.

Statistical Analyses

Data were analyzed using SPSS software using two-way (between groups) ANOVA and one-way (between groups) ANOVA for simple effects analyses followed by planned comparisons (Fisher's LSD) for groups differences with significance at $p < 0.05$.

Results

The effects of CBD on morphine conditioned place preference scores are summarized in Figure 1. Preference scores were near zero in the control group (vehicle + saline) indicating there was little change in baseline and post-conditioning CPP scores. Morphine treated animals showed higher preference scores compared to the control group. Among the saline groups, CBD did not show neither place preference nor aversion. Among the morphine groups, CBD dose-dependently decreased preference scores with a max effect at 10 mg/kg CBD.

A two-way ANOVA revealed a significant main effect for Morphine $F(1,78) = 30.04$, $p < 0.001$. The main effect for Cannabidiol and the Cannabidiol x Morphine interaction were not significant $F(4,78) = 1.57$, $p = 0.19$; $F(4, 78) = 1.68$, $p = 0.16$ respectively. To determine whether morphine possessed place preference, a one-way ANOVA of the Vehicle groups were conducted and revealed a significant effect for Morphine $F(1,15) = 15.69$, $p < 0.001$. To test whether CBD possessed rewarding or aversive properties, a one-way ANOVA among the Saline groups found no significant treatment effect $F(4,39) = 1.21$, $p = 0.32$. In order to determine whether CBD attenuated opioid reward, a one-way ANOVA on morphine groups were performed and found a treatment effect that approached significance $F(4,37) = 2.30$, $p = 0.077$.

Planned comparisons among the morphine groups found 10.0 mg/kg had significantly lower preference scores than the CBD vehicle ($p = 0.033$).

Discussion

The challenge in pain management is to deliver analgesic treatment that is fully efficacious but also void of abuse liability. The present research shows CBD can attenuate opioid reward without producing aversion or reward by itself. These findings suggest that a CBD-opioid formulation may be void of abuse liability and useful in pain management settings.

Although capable of attenuating morphine reward, CBD is not without several translational challenges. Among these may be the poor absorption of CBD when given by enteral administration. Research to enhance CBD bioavailability through chemical modification led to the development of a CBD derivative (cannabidiol mono valine mono hemisuccinate: CBD-val-HS) that possess characteristics that may be useful in clinical populations. This derivative is readily absorbed within 30 minutes of administration and that stable and biologically relevant blood levels persist beyond 12 hrs post administration. Whether this CBD derivative can attenuate rewarding properties of opioids and retain analgesic properties is unknown.

CHAPTER III

EXPERIMENT 1: PLACE PREFERENCE ASSAY

Introduction

The purpose of this study was to identify whether CBD-val-HS can attenuate opioid reward without producing aversion or reward in the CPP paradigm. In this study we selected oxycodone as the opioid probe because it is a more clinically relevant compound in pain management settings and a drug frequently abused among opioid addicts. We predict oxycodone will produce place preference that will be dose-dependently attenuated by CBD-val-HS. Further, we predict CBD-val-HS, when given alone, will not produce place preference nor aversion.

Method

Subjects and apparatus were as described in the pilot study.

Procedure

The groups in this study formed a 2x5 factorial design that combined 2 levels of oxycodone (saline and 3.0 oxycodone) and 5 levels of CBD-val-HS (vehicle and 4 doses). Oxycodone (Tocris, Boston, MA) was dissolved in 0.9% saline to yield a dosage of 3.0 mg/ml. CBD-val-HS 7.0, 8.0, 12.0, 16.0 mg/kg (ELI Laboratories; Oxford, MS) were dissolved in a solution of 5% ethanol/5% cremaphor of injectable water. Mice received sequential dual IP injections of test articles in a volume of 1 ml/kg.

The details of the CPP procedure was as described in the pilot study.

Statistical Analyses

Data were analyzed using SPSS software using two-way (between) ANOVA and one-way (between groups) ANOVA for simple effects followed by planned comparisons with significance at $p < 0.05$.

Results

The effects of CBD-val-HS on oxycodone conditioned place preference scores are summarized in Figure 2. Preference scores were near zero in the control group (vehicle + saline) indicating there was little change between baseline and post-conditioning CPP scores. Oxycodone treated animals showed higher preference scores compared to the control group. Among the saline groups, CBD-val-HS did not show place preference nor aversion. Among the oxycodone groups, CBD-val-HS dose-dependently decreased preference scores with a maximum effect at 8 mg/kg.

A two-way ANOVA of these CPP data revealed a significant main effect for Cannabidiol-val-HS and the Cannabidiol-val-HS x Oxycodone interaction $F(4,129) = 1.203$, $p = 0.025$; $F(4, 129) = 1.541$, $p = 0.32$ respectively. The main effect for Oxycodone was not significant $F(1,129) = 16.331$, $p = 0.337$. To determine whether oxycodone possessed place preference, a one-way ANOVA of the Vehicle groups were conducted and revealed a significant effect for Oxycodone $F(1,24) = 10.784$, $p = 0.003$. To test whether CBD-val-HS possessed rewarding or aversive properties, a one-way ANOVA among the saline groups found no significant treatment effect $F(4,66) = 1.461$, $p = 0.224$. In order to determine whether CBD-val-HS attenuated opioid reward, a one-way ANOVA on oxycodone groups found no significant treatment effect $F(4,63) = 1.22$, $p = 0.310$. Planned comparisons among the oxycodone groups found 8.0 mg/kg CBD-val-HS had significantly lower preference scores than vehicle ($p = 0.033$).

Discussion

The present study sought to determine whether the CBD derivative, CBD-val-HS, could alter the development of oxycodone reward in the condition place preference paradigm. As predicted, 3.0 mg/kg oxycodone produce robust place preference indicative of reward. This finding is consistent with literature demonstrating opioids such as morphine produce preference in the CPP paradigm (Prus et al., 2009).

CBD-val-HS attenuated the rewarding effects of oxycodone place preference with a dose of 8.0 mg/kg. Further, this novel derivative itself is void of reward or aversive properties. This finding is consistent with previous research in this lab demonstrating CBD can alter morphine place preference and void of an aversive effect. Although the mechanism of action is unknown, the behavioral effects of CBD-val-HS in this model resemble that of CBD and strongly argue that these compounds carry out the same mechanism of action.

Taken together, results from these CPP studies align with reports that CBD also blocks opioid reward in the ICSS paradigm (Katsidoni et al., 2013) and a literature that CBD itself lacks hedonic or aversive actions (Mechoulam et al., 2002; Mechoulam et al., 2007; Parker et al., 2004). These findings suggest CBD-val-HS may translate well as a useful pharmacotherapy in preventing substance abuse. Further, the absence of of psychotomimetic properties of CBD-val-HS should abate concerns raised by substitution therapies like methadone maintenance programs.

Much of today's opioid epidemic is attributed to overuse of prescription opioids in pain management and CBD-val-HS may also show efficacy here as an abuse deterrent in a dual drug formulation. Indeed, few alternatives exist for effective pain management outside of opioids and there are attempts to develop dual drug formulations that mitigate opioid reward while maintaining the full analgesic profile (Townsend et al., 2017). There is evidence to suggest that a

CBD opioid formulation would possess such an analgesic profile. Neelakantan *et al.* (2015) reported that a CBD-opioid combination possesses synergistic analgesia in the abdominal writhing assay. Whether a CBD-val-HS + opioid formulation possess robust analgesia across a range of nociceptive assays remains to be determined.

CHAPTER IV

EXPERIMENT 2: NOCICEPTIVE ASSAYS

Introduction

Like non-opioid therapies, the biggest challenge for CB compounds as analgesics is in their modest efficacy. Combination of opioid and CB receptor agonists has shown synergistic effects in a number of pain assays. This synergistic effect is thought to be mediated by signaling interactions of CB1 and mu-opioid receptors co-expressed in brain structures that modulate nociceptive responses (Wilson-Poe et al., 2008; Hall et al., 2005; Mansour et al., 1988) through a descending pain control circuit (Basbaum et al., 1984). Neelkatantan et al., demonstrated combinations of CBD and morphine could produce synergistic analgesic effects in a murine abdominal writhing assay. However, these combinations have produced sub-additive nociceptive responses in models of thermal nociception (Neelaktantan et al., 2014).

Studies in this laboratory have shown that CBD in combination with a sub-analgesic dose of morphine can produce synergistic analgesic effects on tactile allodynia in murine model of cisplatin induced neuropathy (CIN). Further studies in this CIN model have shown that 1) CBD-val-HS in combination with a sub-analgesic dose of morphine and 2) CBD-val-HS administered alone can produce robust pain relief equivalent to a fully efficacious dose of morphine.

Whether CBD-val-HS acts and interacts with opioids in a manner similar to CBD in the aforementioned thermal and inflammatory nociceptive assays is unknown. Thus, the next set of

experiments examined the analgesic properties of CBD-val-HS given alone and in combination with increasing doses of oxycodone on the hotplate and abdominal writhing assays.

Method

Subjects

Animal characteristics and housing conditions were as described earlier.

Procedure

These experiments formed a 2x3 factorial design which entailed two levels of CBD-val-HS (cremaphor and 8.0 mg/kg) and three levels of oxycodone (saline, 1.0 and 3.0 mg/kg oxycodone) with 9-17 animals per experimental condition. Oxycodone (Tocris, Boston, MA) was dissolved into saline the first day of testing. CBD-val-HS 8.0 mg/kg (prepared by ELI laboratories; Oxford, MS) was kept refrigerated and brought to room temperature prior to administration. Mice received sequential dual IP injections of test articles in a volume of 1 ml/kg.

A hotplate apparatus (Harvard Instruments, Model #52-8570) was used to quantify thermal nociception. This consisted of an open top acrylic enclosure (12.7 X 15.24 cm) positioned on a plate heated set to 52^{o}C. A digital timer operated via a foot switch measured the latency of a nociceptive response (i.e., hind paw flutter, lick, or an escape response) and presented on a digital display. Mice were given IP injections of test articles and transported to the testing room 30 minutes prior to behavioral testing for acclimation to the experiment room. Mice were then placed onto the hotplate and immediately removed following a nociceptive response or after a 45 second cut-off to prevent tissue damage. Following thermal nociceptive testing, animals were given a no-drug/test-free week then enrolled into acetic acid writhing assay.

Acetic acid writhing testing was used to quantify inflammatory nociception. Testing was conducted in clear, open-top, acrylic observation chambers (12.7 X 15.24 cm) located on a smooth surface. Mice were given IP injections of test articles and transported to the testing room 30 minutes prior to behavioral testing for acclimation to the experiment room. For testing, mice were then given an IP injection of 0.7% acetic acid in a volume of 10 ml/1kg and immediately placed in an observation chamber for 30 minute test. The number of abdominal stretches served as the dependent measure. Following testing, animals were returned to home cage and euthanized via Euthasol at the completion of the experimental test session.

Statistical Analyses

Data was analyzed using SPSS software using two-way (between group) ANOVA and one-way (between groups) ANOVA for simple effects analyses followed by planned comparisons for group differences with significance at $p < 0 .05$. In case of unequal variances (assessed by Levene's test), analyses were performed on square-root transformed count data. If main effects or interactions were significant, data were further analyzed by one-way ANOVA and Fisher's LSD post hoc tests.

Previous research has reported that around 16% of mice do not respond to acetic acid and 23% of mice display one writhe after a sham injection (Collier et al., 1968). Data was screened for non-responders and outliers prior to analysis. This amounted to the removal of 2 non-responders per group in addition to removing animals with less than 5 writhes.

Results

The effects of oxycodone and CBD-val-HS on hotplate responses are summarized in Figure 3. Vehicle and the sub-analgesic dose of oxycodone (1.0 mg/kg) did not affect hotplate responses whereas the 3.0 mg/kg oxycodone produced robust analgesia demonstrated with high

response latencies. CBD-val-HS alone produced response latencies equivalent to 3.0 mg/kg Oxycodone. Further, the sub-analgesic and analgesic doses of oxycodone given in combination with CBD-val-HS produced sub-additive effects on hotplate latencies.

Consistent with these observations, a two-way ANOVA performed on these data revealed a significant main effect for Oxycodone, $F(2,76)= 3.830$, $p = 0.026$ and a significant CBD-val-HS x Oxycodone interaction $F(2,76)= 5.761$, $p = 0.005$. The CBD-val-HS term was not significant $F(1,76)= 0.49$, $p = 0.619$. To determine which oxycodone dose produced analgesia, a one-way ANOVA of the vehicle groups were conducted and revealed a significant effect for Oxycodone $F(2,40)= 6.467$, $p = 0.004$. Post hoc analyses among the cremaphor groups found 3.0 mg/kg oxycodone produced significantly higher hotplate response latencies than saline and 1.0 mg/kg oxycodone ($p = 0.001$). To test whether CBD-val-HS produced analgesia, a one-way ANOVA among the saline groups was conducted and revealed a significant main effect for CBD-val-HS $F(1,35) = 6.273$, $p = 0.017$.

To determine whether CBD enhanced oxycodone analgesia, a one way on the CBD-val-HS groups were conducted and revealed a significant main effect $F(2,36) = 3.674$, $p = 0.035$. Post hoc analysis among these groups revealed 8.0 mg/kg CBD-val-HS + 1.0 mg/kg oyxcodone produced a significantly lower hotplate response latency than 8.0 mg/kg CBD-val-HS ($p =0.010$). There was no significant difference between 8.0 mg/kg CBD-val-HS and 8.0 mg/kg CBD-val-HS+ 3.0 mg/kg oxycodone ($p > 0.230$).

The effects of oxycodone and CBD-val-HS on abdominal writhing responses are summarized in Figure 4. Oxycodone produced a dose-dependent decrease in writhing response indicative of analgesia. In the Saline treated groups, CBD-val-HS also attenuated writhing illustrating this CB derivative possesses analgesic properties against inflammatory nociception.

CBD-val-HS in combination with increasing doses of oxycodone appears to produce additive effects in attenuating abdominal writhes.

A two-way ANOVA was carried out, and as Levene's test for equality of variances was significant, data were transformed using log square root. This analysis revealed a significant main effect for Oxycodone, $F(2,52)=22.939$, $p<0.001$ and a significant main effect for CBD-val-HS $F(2,52)=46.082$, $p<0.001$. The CBD-val-HS x Oxycodone interaction term was not significant $F(2,52)= 0.440$, $p = 0.646$. To determine which oxycodone dose decreased abdominal writhes, a one-way ANOVA of the vehicle groups were conducted and revealed a significant main effect for Oxycodone $F(2 ,25)= 23.534$, $p<0.001$. Post hoc analyses among these groups found 3.0 mg/kg oxycodone produced significantly lower abdominal writhes than saline and 1.0 mg/kg Oxycodone ($p<0.001$). To test whether CBD-val-HS produced decreased writhing, a one-way ANOVA among the saline groups was conducted and revealed a significant main effect for CBD-val-HS $F(1,19) = 5.943$, $p =0.025$.

To determine whether CBD enhanced oxycodone analgesia, a one way on the CBD-val-HS groups were conducted and revealed a significant effect for CBD-val-HS $F(2,27) =23.454$, $p<0.001$. Post hoc analysis among these groups demonstrated 3.0 mg/kg oxycodone + CBD-val-HS significantly decreased abdominal writhing ($p<0.001$).

Discussion

Experiments 2 and 3 sought to determine whether a dose of CBD-val-HS that blocks opioid reward administered alone or in combination with a sub-analgesic or analgesic doses of oxycodone would affect nociceptive processes in the hotplate and abdominal writhing assays.

As expected, 3.0 mg/kg oxycodone was robust in increasing hotplate response latency and decreasing abdominal writhing responses while 1.0 mg/kg oxycodone was ineffective in both assays. These responses are consistent with previous literature demonstrating high dose

opioids such as oxycodone produce analgesic responses in both supraspinal thermal assays as well as peripheral inflammatory pain (Watson et al., 2003; Yao et al., 2012). CBD-val-HS alone produced analgesic effects in both assays but was most effective when compared to oxycodone against thermal nociception. Interestingly, there was a differential interaction of CBD-val-HS and oxycodone across the two nociceptive assays. Subadditive responses were observed in the hotplate assay while additive responses were observed in the abdominal writhing assay.

Data from the hotplate assay are consistent with Neelkanatan et al., who demonstrated CBD in combination with an opioid (morphine) produce subadditive effects (2015). Unlike CBD, CBD-val-HS produced robust analgesia equivalent to high dose Oxycodone. These responses are most likely due to a superior absorption profile allowing binding to pain regulating sites include the periaqueductal gray (PAG), thalamus, amygdala, spinal cord and/or the peripheral nervous system which modulates inflammatory pain by affecting factors involved in inflammation (Rahn and Hohmann, 2009; Chiou; Zogopoulos et al., 2008; Pertwee, 2001).

CHAPTER V

GENERAL DISCUSSION

Opioid use for chronic pain syndromes has seen significant growth in the last several decades. The perception that newer opioids possessed little abuse liability in pain management settings has led to the opioid abuse crisis we experience today (Kaye et al., 2017). These opioids in various novel formulations were marketed to physicians as non-addictive without clear and compelling evidence. It is estimated that of the 60% overdose deaths in the United States in 2014, over 28,647 can be linked to an opioid prescription to treat pain (CDC, 2016). Currently, several states have or are in the process of filing lawsuits against Purdue Pharma and other pharmaceutical companies for false marketing (Semeuls, 2017) in an attempt to recover associated costs of opioid abuse. While there are continued attempts to formulate full efficacy opioids void of abuse liability, these efforts have met with little success (Moorman-Li et al., 2012; Pappagallo & Sokolowska, 2012; Rappaport, 2008; Katz, 2008). The work herein demonstrates in a preclinical model that a cannabidiol derivative + oxycodone combination is formulation that would achieve high efficacy in treating pain yet be void of abuse liability.

One additional finding from this research was that cannabidiol-val-HS possesses significant analgesic properties against acute thermal and persistent inflammatory nociception. This work aligns well with earlier work form this laboratory that shows CBD-val-HS possess analgesic activity in a murine model of chemotherapy induced neuropathy (CIN; Harris, 2017).

Against acute thermal nociception and CIN, CBD-val-HS analgesic effects are as efficacious as oxycodone and morphine, respectively. Collectively, these studies suggest it may be unnecessary to rely on an opioid x CBD-val-HS formulation to treat certain pain conditions. An important unanswered question is whether CBD-val-HS possesses analgesic activity across a broad range of other pain conditions including, among others, arthritic, cancer, and migraine models.

There are a number of additional research questions that would facilitate the movement of this compound through the drug discovery pipeline and enter into clinical trials. The first step would entail experimental designs to perform isobolographic analyses. Isobolograms are the gold standard in studying drug-x-drug interactions and determine whether a compound possesses subadditive, additive, or synergistic properties. These data identify relevant doses of each compound necessary to achieve full clinical efficacy. As certain opioids may be more efficacious against specific pain conditions, it may be necessary to perform isobolograms across a broad range of opioids with this novel CBD derivative to create a set of novel formulations tailored to treating a wide variety of chronic pain conditions.

As with any novel therapeutic, its use may be limited by adverse side effects. Future research should explore the possibility that a CBD-val-HS formulation with opioids produce undesirable effects that might limit its use. While the current research demonstrates the most serious issues of opioid addiction is mitigated by CBD-val-HS, it will be important to test this formulation in assays that assess sedation, ataxia, respiratory depression, and other physiological side effects. We believe it is unlikely that CBD-val-HS as a stand-alone analgesic will possess such characteristics as its parent molecule CBD does not show any adverse side effects (America, 1998). Indeed, CBD has demonstrated to be safe and effective for use in epilepsy and is in current use today for this debilitating condition. We assume this modest chemical

variation that enhances bioavailability will possess a similar safety and efficacy profile.

Perhaps the most intriguing scientific question unanswered is how CBD-val-HS acts on nervous system targets that differentially modulates both nociception and addiction. Little is known about the CNS action of CBD but the patterns of CBD-val-HS show similar effects on a wide variety of behavioral endpoints. A full screen across CNS receptors may reveal CBD-val-HS has selectivity to a number of non-CB receptors or, perhaps, lack receptor selectivity altogether. Future mechanism of action studies may show that CBD-val-HS acts on nervous system activity that interfere with intracellular communication process that maintains normal neuronal functioning. Regardless of the exact mechanism of action, that a CBD-val-HS x opioid formulation can interfere with reward processes while enhancing analgesia represent a significant turning point in the opioid abuse crisis today.

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LIST OF APPENDICES

APPENDIX A: FIGURES AND CAPTIONS

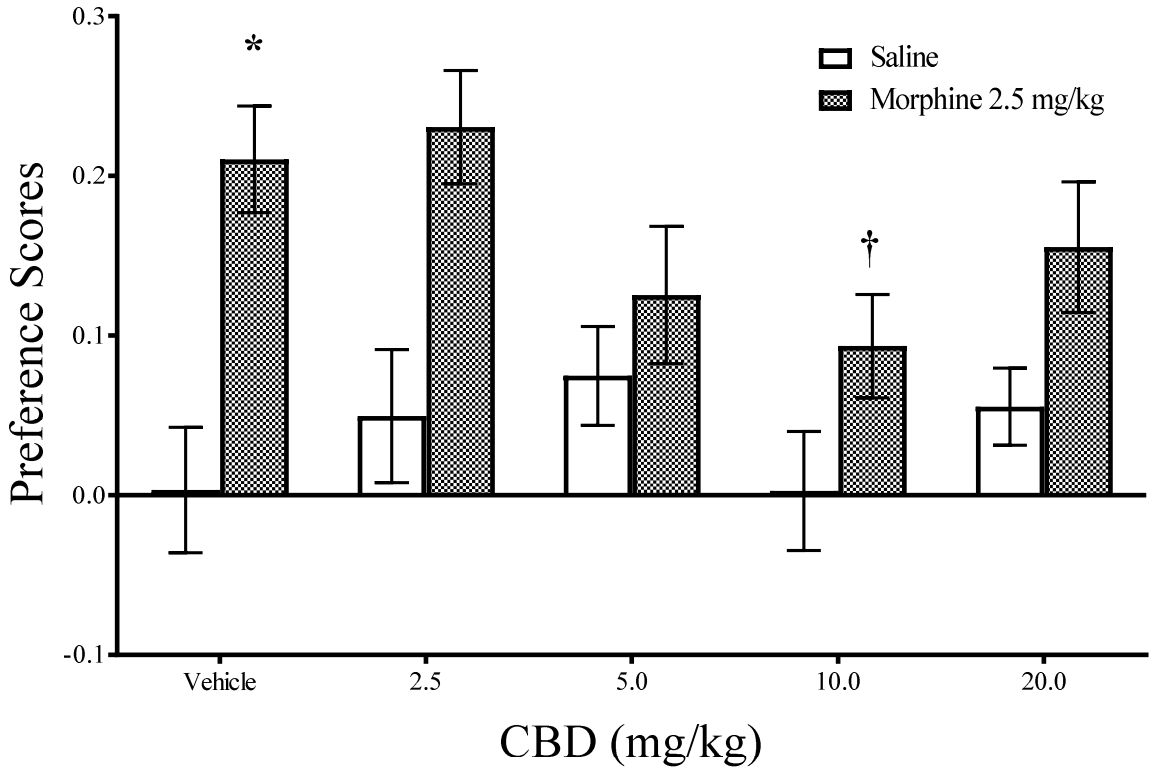

Figure 1. The effects of CBD on morphine place preference scores. Values represent difference in the mean ratio of time (seconds) spent in the S+ (drug-paired) chamber during pre- and post-condition trials. Open bars reflect saline treated animals and striped bars represent morphine treated animals. *denotes significant difference form the vehicle group. † denotes significant attenuation of morphine preference. Sample sizes were n= 7-10.

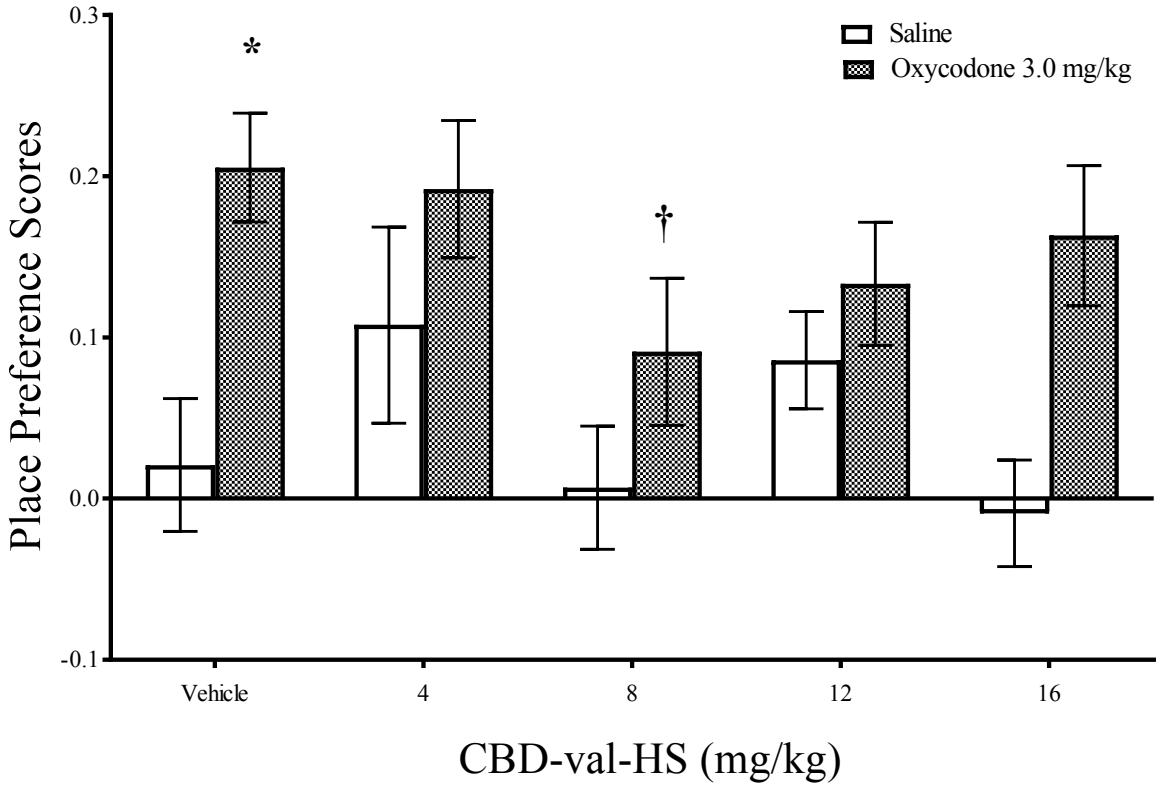

Figure 2. The effects of CBD-val-HS on oxycodone place preference scores. Values represent difference in mean ratio of time (seconds) spent in the S+ (drug-paired) chamber during pre-and post- condition trials. Opens bars reflect saline treated animals and hatched bars represent oxycodone treated animals. * denotes significant difference from the vehicle group. † denotes significant attenuation of oxycodone preference. Sample sizes were n = 11-15.

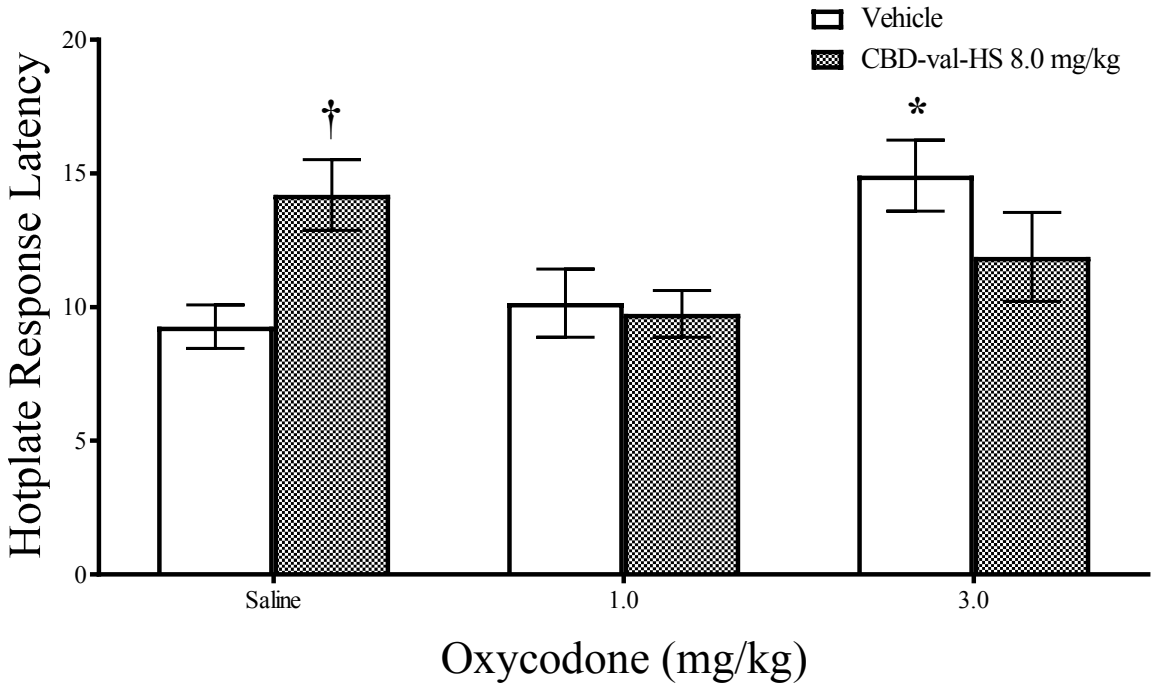

Figure 3. The effects of CBD-val-HS and oxycodone on hotplate response latencies. Values represent the mean latency (seconds) of a hind-paw lick or flutter. * denotes a significant difference from the saline group. Sample sizes were n= 9-17.

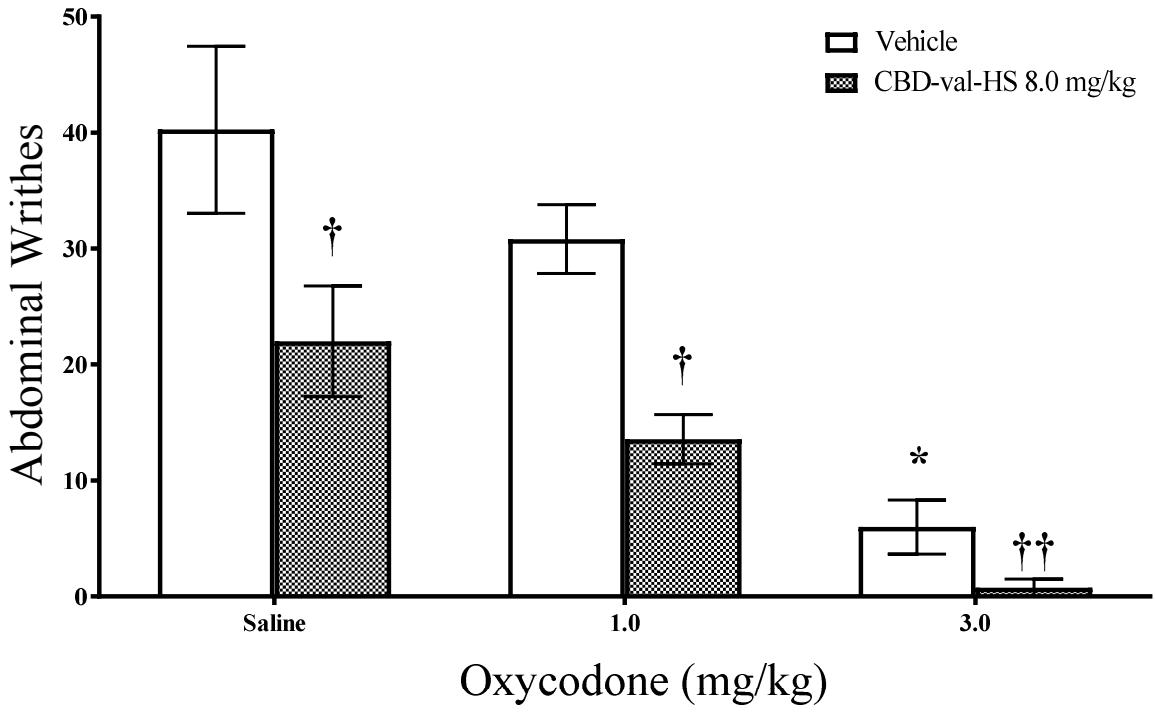

Figure 4. The effects of CBD-val-HS and oxycodone in the abdominal writhing test. Values represent the mean number of writhes following an intraperitoneal injection of 0.7 % acetic acid over a 30 minute test session. * denotes a significant difference from the saline group. † denotes a significant difference from oxycodone 3.0 mg/kg. Sample sizes were n= 8-15.

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EDUCATION

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Thesis: Cannabinoid Modulation of Cisplatin-Induced Neuropathy

Ph.D. — August 2017
Behavioral Neuroscience
University of Mississippi, University, MS
Dissertation: Differential Modulation of Oxycodone Reward and Analgesia by a Cannabinoid Derivative

PROFESSIONAL AFFILIATIONS

Society for Neuroscience

RESEARCH INTERESTS

Development, validation, and utilization of animal models (chronic pain and analgesia)
Drug efficacy screening (analgesics)

AWARDS

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Class Marshall Doctoral Hooding Ceremony
2017

RESEARCH PUBLICATION

Harris HM, Gul W, ElSohly MA, Sufka KJ. (2016). Cannabinoid modulation of cisplatin induced neuropathy in mice. *Planta Medica*, 82, 1169-1182.

Harris HM, Carpenter JM, Black JR, Smitherman TA, Sufka KJ. (2017). The effects of repeated nitroglycerin administrations in rats; modelling migraine-related endpoints and chronification. *Journal of Neuroscience Methods.*

Markos JR, Harris HM, Gul W, ElSohly MA, Sufka KJ. (2017). Effects of cannabidiol on morphine conditioned place preference in mice. *Planta Medica*.

MANUSCRIPTS UNDER REVIEW, SUBMITTED, OR IN PREPARATION

Markos JR, Harris HM, Gul W, ElSohly MA, Sufka KJ. (Submitted). Effects of cannabidiol on morphine conditioned place preference in mice. *Planta Medica*

Harris HM, Gul W, ElSohly MA, Sufka KJ. (in preparation). Effects of cannabidiol and a novel cannabidiol analog against tactile allodynia in a murine model of cisplatin-induced neuropathy; synergistic effects of sub-analgesic doses or morphine.

Cucinello J, Warren J, Oellerich P, Harris HM, Sufka KJ. (in preparation). Enhancing translational relevance in a murine model of cisplatin-induced neuropathy using a novel arena apparatus.

INTERNATIONAL CONFERENCES PAPERS AND ABSTRACTS

Harris HM, Gul W, ElSohly MA, Sufka KJ (November 2017) Analgesic effects of cannabidiol and a novel cannabidiol analog in a murine model of cisplatin-induced neuropathy; synergistic effects with sub-analgesic doses of morphine. Society for Neuroscience, Washington, DC.

Markos JR, Harris HM, Gul W, ElSohly MA, Sufka KJ (November 2017) Cannabidiol blocks morphine place preference in mice. Society for Neuroscience, Washington, DC.

Harris HM, Carpenter JM, Black JR, Smitherman TA, Sufka KJ. (November 2016). Further validation of a nitroglycerin-induced episode migraine model in rats. Society for Neuroscience, San Diego, CA.

Harris HM, Sufka KJ, Gul W, ElSohly MA. (November 2014). THC and CBD attenuate but do not prevent cisplatin neuropathy in mice. Society for Neuroscience, Washington,D.C.

REGIONAL AND LOCAL CONFERENCE PAPERS AND ABSTRACTS

Cucinello J, Warren J, Oellerich P, Harris HM, Sufka KJ. Evoked vs spontaneous tactile allodynia: enhancing the translational relevance in a murine CIN model. (2017). UMMC Annual Neuroscience Research Day.

Markos JR, Harris HM, Gul W, ElSohly MA, Sufka KJ. The effects of cannabidiol on morphine conditioned place preference in mice. (2016). Annual UM Neuroscience Minor Research Showcase.

Harris HM, Craig HK, Ali Z, Abe N, Khan IA, Sufka KJ. (July 2014). Rewarding and antinociceptive properties of Mitragynine speciosa products in rats. Foods and Veterinary Medicine Science and Research Conference.

Craig HK, Harris HM, Ali Z, Abe N, Khan IA, Sufka KJ. Evaluation of Sceletium tortuosum products on conditioned place preference and hotplate paradigms in rats. (July 2014). Rewarding and antinociceptive properties of Mitragynine speciosa products in rats. Foods and Veterinary Medicine Science and Research Conference.

Loria MK, Harris HM, Lewellyn K, Zjawiony JK, Ali Z, Khan IA, Sufka KJ. (April 2013). Evaluating preference-seeking and aversive qualities of salvia divinorum and mitragyna speciosa. 12th Annual Oxford International Conference on the Science of Botanicals.

PRESENTATIONS

Cannabinoid modulation of cisplatin induced neuropathy. (October, 2013). 3 Minute Thesis Competition, Oxford, MS

GRANTS AND CONTRACTS

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Graduate research assistant and project coordinator: Cannabinoid modulation of opioid-reward and analgesia, Nemus Biosciences, Costa Mesa, CA (02/01/17-07-31-17 Kenneth J. Sufka, Ph.D., PI).

PATENT FILING

Biologically Active Cannabidiol Analogs (serial no. PCT/US17/15366), filed January 27, 2017

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Blake A. Sowers, Department of Biology, Honors College
Thesis: Modeling migraine chronification and its relief; the effects of THC on recurrent migraine endpoints in rats (2016-2017)

Jessica Cucinello, Jontae Warren, Patrick Oellerich, Department of Psychology and School of Pharmacy, Provost sponsored project
Research: Evoked and spontaneous allodynia in a murine model of cisplatin induced neuropathy (2017)

Margaret Rousseau, Department of Biology, Honors College
Thesis: In progress