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Lorcaserin as a potential opioid-sparing adjunct

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LORCASERIN AS A POTENTIAL OPIOID-SPARING AJDUNCT

A dissertation submitted in partial fulfillment of the requirements for the degree of Doctor in Philosophy at Virginia Commonwealth University.

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"It takes a village to raise a scientist."

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Chapter 1 – Introduction

Chapter 2 – Characterization of the pharmacology of lorcaserin and its effects on acute opioid-induced antinociception

Chapter 3 - The Effect of Lorcaserin on the Development of Opioid Tolerance

List of Abbreviations

5-HT	Serotonin
5-HT$_{1A}$	Serotonin type-1A receptor
5-HT$_{1B}$	Serotonin type-1B receptor
5-HT$_{2A}$	Serotonin type-2A receptor
5-HT$_{2B}$	Serotonin type-2B receptor
5-HT$_{2C}$	Serotonin type-2C receptor
b.i.d.	Twice per day
CFA	Conjugated Freud's adjuvant
CL	Confidence Limits
CNS	Central nervous system
CYP	Cytochrome P450 enzyme
DAMGO	([D-Ala2, N-MePhe4, Gly-ol]-enkephalin)
DMEM	Dulbecco's Modified Eagle's Medium
DOR	Delta (δ)-Opioid Receptor
DRG	Dorsal root ganglion
EC$_{50}$	Effective concentration (half-maximal)
ED$_{50}$	Effective dose (half-maximal)
E$_{Max}$	Maximal effect
GABA	gamma-Aminobutyric acid
GPCR	G-Protein Coupled Receptor
GRK	G-protein coupled Receptor Kinase
HBSS	Hanks Balanced Salt Solution

i.c.v.	Intracerebroventricular injection
i.p.	Intraperitoneal injection
i.t.	Intrathecal injection
KO	Knockout
KOR	Kappa (κ)-Opioid Receptor
LSD	Lysergic acid diethylamide
MOR	Mu (μ)-Opioid Receptor
MPE	Maximum Possible Effect
mRNA	Messenger ribonucleic acid
NSAID	Non-steroidal anti-inflammatory
p.o.	By mouth, orally
PAG	Periaqueductal Grey
PI	Phosphoinositol
PKC	Protein Kinase C
PLC	Protein Lipase C
RVM	Rostral Ventral Medulla
s.c.	Subcutaneous injection
SSRI	Selective serotonin reuptake inhibitors
TCA	Tricyclic antidepressants
WT	Wild-type

Abstract

LORCASERIN AS A POTENTIAL OPIOID SPARING ADJUNCT

By Kumiko Marie Lippold

A dissertation submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy at Virginia Commonwealth University.

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Opioids, such as oxycodone, morphine, and fentanyl, are commonly used medications in the treatment of moderate to severe pain. In spite of their efficacious analgesic properties, their increased prescribing rates by physicians and inherent abuse-related effects have led to the ongoing opioid epidemic. Their clinical utility is limited by the risk of adverse dose-dependent side effects, such as constipation and respiratory depression, and the development of tolerance and dependence. Opioid-sparing adjunctive therapies are sought to address these issues by reducing the dose of opioid needed to achieve analgesia through alternative non-opioidergic mechanisms and as a result, reduce the incidence of the previously mentioned side effects. Serotonin type-2C receptor agonists have demonstrated antinociceptive efficacy in preclinical models of chronic pain. Lorcaserin is a selective 5-HT$_{2C}$ receptor agonist and was reported to attenuate the abuse-related effects of oxycodone. The antinociceptive properties of 5-HT$_{2C}$ receptor agonists and their potential to alter the abuse-related effects of commonly abused drugs suggest that lorcaserin may be a potential opioid-sparing therapeutic. The goal of these studies was to evaluate the utility of

lorcaserin, in combination with opioids, in a preclinical model of acute pain. Based on previous studies demonstrating the antinociceptive activity of 5-HT$_{2C}$ agonists, the hypotheses for these studies were that lorcaserin would increase the acute antinociceptive effects of opioids and would attenuate the development of tolerance associated with chronic opioid consumption.

The results demonstrate that the acute antinociceptive effects and the time-course of activity of opioids were enhanced by doses of lorcaserin. These effects were mediated through activation of the 5-HT$_{2C}$ receptor and were not blocked by administration of naloxone. Additionally, the acute effects of lorcaserin to increase opioid potency and time course was not mediated through changes in opioid distribution in the blood or central tissues.

Opioid tolerance was evaluated *in vivo*, and tolerance was developed using two methods of treatment: an acute (single dose administration) model of tolerance and a multiple-injection model. Testing the effect of lorcaserin in these models was important because current research suggests that the mechanisms that underlie both models of tolerance are distinct from one another. The results demonstrate that lorcaserin significantly blocked the development of acute tolerance in the whole animal and on a single cell level in dorsal root ganglion cell cultures.

In the multiple-day tolerance model, lorcaserin partially attenuated the development of opioid antinociceptive tolerance. Chronic administration of an opioid is associated with desensitization of the MOR, and the effect of lorcaserin on opioid tolerance may be mediated through changes in MOR functional activity. Upon further investigation using agonist-stimulated [^{35}S]GTPγS, the results showed that lorcaserin altered basal binding of [^{35}S]GTPγS but not agonist-stimulated binding in mice that received chronic opioid treatment. These data suggest that the effect of lorcaserin on opioid tolerance, in the multiple-injection model, is not mediated through changes in MOR functional activity. Collectively, the tolerance studies suggest that the effect of 5-HT$_{2C}$

receptor activation by lorcaserin has differential effects on the stages of opioid tolerances and further supports the notion that the mechanisms that underlie the stages of opioid tolerance are distinct. Given the efficacy of lorcaserin to increase the acute antinociceptive effects of opioids and its ability to impair the development of opioid tolerance, collectively, these data suggest that lorcaserin may be a useful opioid-sparing adjunctive therapy.

I. Introduction

“Not the Opium-eater, but the opium, is the true hero of the tale, and the legitimate centre on which the interest revolves.”

-Thomas De Quincey, Confessions of an English Opium Eater

The earliest reference to opiate use dates back to 3400 B.C by the ancient Sumerians, who cultivated the opium poppy plant (*Papaver somniferum*), or the “Joy Plant” as it was referred to, in lower Mesopotamia (Brownstein, 1993). It is suggested that opium spread to the rest of the old world from its early origins in Sumeria and since then, opium and its subsequent derivates have remained mainstay for both therapeutic and recreational purposes. From early autobiographical documentation in Confessions of an English Opium Eater by Thomas De Quincey, to frequent references in pop culture and music, to our modern day opioid epidemic, opiates are unyielding in their captivation.

Opium is comprised of several alkaloid compounds called opiates and from this material in 1805, a German pharmacist named Friedrich Sertürner isolated the first active alkaloid from the opium poppy plant (Sertürner, 1805, 1806, 1817). He named this compound “morphine” after the Greek God, Morpheus, as it had a tendency to induce sleep. Following the invention of the hypodermic syringe and needle, morphine gained popularity as a treatment for pain in surgical procedures and as an anesthetic adjunct (Wood, 1858; Hunter, 1863; Hamilton and Baskett, 2000). Morphine, though efficacious for the treatment of pain, was still not safe for use due to its abuse potential and side effects. As a result, a great deal of time was spent on developing a safer and non-addicting opiate and in 1898, this search yielded heroin. Heroin was claimed to be free of abuse liability and was more potent than morphine (Brownstein, 1993). Heroin was marketed by Bayer as a morphine substitute and as a cough suppressant for children until its addictive nature was realized (United Nations Office on Drugs and Crime, 1953).

In spite of failed attempts at developing a "safer" opiate, the search for the holy grail of opiate drugs continued and led to the subsequent synthesis of one of the most well-known prescription opioids in 1917: oxycodone (Falk, 1917). The term "opiate" refers to compounds that are derived from, and are structurally similar to, naturally occurring opium compounds and this encompasses alkaloids such as thebaine, morphine, heroin, and also oxycodone (Rosenblum *et al.*, 2008). Oxycodone was the first of many semi-synthetic opioid compounds that are structurally similar to morphine and contain a similar structural backbone (Figure 1.1). The term "opioid" represents a broad class of compounds that have morphine-like activity but may be structurally similar or dissimilar to traditional opiates and as a result, may be either naturally occurring or synthesized (Rosenblum *et al.*, 2008). Semi-synthetic compounds, such as fentanyl and methadone, fall under the 'opioid" category because they exhibit opiate-like activity but are structurally distinct from morphine (Figure 1.1).

Decades after the initial synthesis of oxycodone in the mid-1990s, oxycodone was marketed by Purdue Pharma under the name "Oxycontin" as a safer opioid analgesic for the treatment of acute and chronic pain (Van Zee, 2009). Over the next decade, prescription opioid sales quadrupled from 1994 to 2014 because of the importance of providing "pain management" (Haddox *et al.*, 1997; CDC, 2017). In the midst of the widespread opioid prescribing, there was a simultaneous increase in the non-medical use of these opioids (US Government Accountability Office, 2011; Hughes *et al.*, 2016). The consequences of these prescribing rates were widespread, with the CDC estimating the nearly 1.9 million Americans qualify as having an opioid use disorder and reporting that approximately 115 individuals experience fatal overdose every day (CDC *et al.*, 2016).

In an effort to address the opioid epidemic, the United States Government drafted a five-part plan that involved improving our understanding of the physiology of pain and developing alternative treatments for pain that do not rely on opioidergic mechanisms (F Collins *et al.*, 2017). Pain was endorsed as a the “fifth vital sign” by the American Pain Society and until recently, opioids were the mainstay for treating these conditions (Max *et al.*, 1995). The reality is that although opioids provide adequate pain relief for some conditions but do so at a risk. The risks of opioid use are great and chronic use is associated with an increased risk in unwanted side effects, such as constipation, dependence, and an overall increase in opioid-related mortality (Gomes *et al.*, 2011).

Several avenues of opioid-sparing medications have been explored, including non-steroidal anti-inflammatories (NSAIDs), gabapentenoids, and antidepressants (Sunshine *et al.*, 1993; Kolesnikov *et al.*, 2003; Nikolajsen *et al.*, 2006; Derry *et al.*, 2009, 2013; Gaskell *et al.*, 2009; Straube *et al.*, 2010; Wibbenmeyer *et al.*, 2014; Sullivan *et al.*, 2016). Each category provides its own set of risks and benefits and vary overall in their efficacy in treating pain. NSAIDs and prescription opioid combinations, however, have found great success in reducing the overall dose of opioid needed to treat pain but their chronic use has significant gastrointestinal side effects (Gaskell *et al.*, 2009; Derry *et al.*, 2013). The varieties of pain in clinical populations require alternative avenues for its treatment, as no two conditions or patients are alike, and the goal of this dissertation is to explore one such alternative mechanism through which the therapeutic effects of opioids can be favorably enhanced

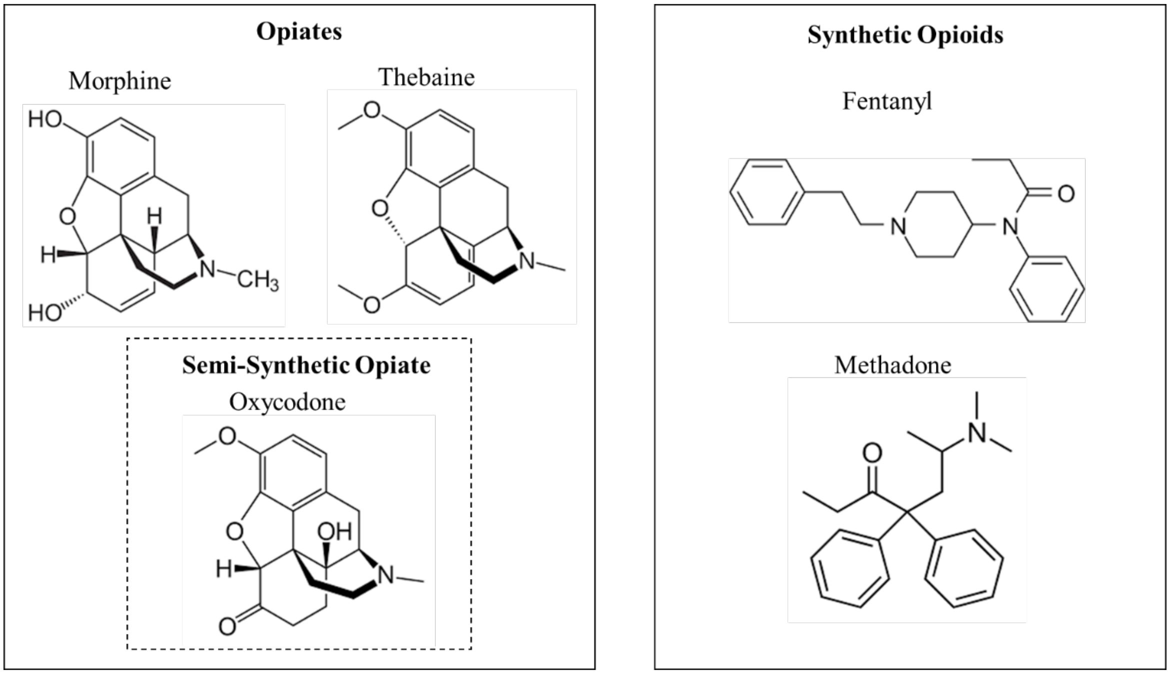

Figure 1.1: Structural characteristics of natural opiate and synthetic opioid compounds. Morphine and thebaine are natural opiate compounds that were isolated from the opium poppy plant (*Papever somniferum*). Oxycodone is a semi-synthetic opiate compound that is derived from a thebaine backbone. Fentanyl and methadone are both synthetic opioids that were not derived from naturally occurring opiate substances but demonstrate opiate-like effects.

II. Opioid Pharmacology

The hypothesis that opiates and similarly derived compounds shared a common binding site was a concept that developed in the mid-1900s (Beckett and Casy, 1954; Portoghese, 1966). With the development of amazingly potent opiates and highly selective antagonists, the notion that these compounds exhibited strict structure-activity relationships favored the existence of specific receptors and in the 1970s, the existence of an opioid receptor was confirmed and a new age of modern opioid pharmacology was ushered in (Goldstein *et al.*, 1971; Pert *et al.*, 1973; Simon *et al.*, 1973). Following these fundamental demonstrations of opioid-receptor mediated activity, the existence of not only one, but multiple, opioid receptors were postulated to exist. A lack of homogeneity among these opioid receptors was presented by Gilbert and Martin in 1976. Several groups identified these distinct subclasses of opioid receptors, which are comprised of the mu-opioid receptor (MOR, μ) (Chang and Cuatrecasas, 1979), the delta opioid receptor (DOR, δ) (Kosterlitz, 1980) and the kappa opioid receptor (KOR, κ) (Gilbert and Martin, 1976; Chang *et al.*, 1979; Schulz *et al.*, 1980). An additional opioid receptor subtype was identified in 1994 by three independent laboratories, and this receptor was identified as the nociceptin/orphanin (n/OFQ) receptor (NOP) (Chang *et al.*, 1979; Bunzow *et al.*, 1994; Fukuda *et al.*, 1994; JB Wang *et al.*, 1994; Mollereau *et al.*, 1994). The endogenous agonist for the NOP receptor, orphanin FQ or nociceptin, antagonizes opioid-mediated antinociception and is considered to be the “anti-opioid” peptide (Mogil *et al.*, 1996). Further discussion of the NOP receptor is not relevant to these studies and is beyond the scope of this dissertation.

These receptors were eventually discovered to be the targets of an endogenous opioid system, comprised of peptidergic compounds with varying affinities for each opioid receptor subtype. The search for endogenous opiates led to the discovery of three general classes: enkephalins (Hughes *et al.*, 1975), endorphins (Cox *et al.*, 1976) and dynorphin (Goldstein *et al.*, 1979) and are each the products of precursor peptides: proenkephalin, proopiomelanocortin and prodynorphin. Additional endogenous opioid peptides have been identified and include: endomorphins and the previously mentioned nociception/orphanin peptides (Mogil *et al.*, 1996; Hackler *et al.*, 1997; Zadina *et al.*, 1997).

Endogenous opioids and exogenous opioids, such as morphine or oxycodone, exert their pharmacological effects through the classical opioid receptors described earlier. MOR, KOR, and DOR share several characteristics and collectively belong to the G protein-coupled receptors (GPCR) superfamily, more specifically of the Gi/o-subtype. They exhibit the typical seven transmembrane regions with an extracellular NH_2 terminus and an intracellular COOH terminus and display ~60% sequence homology with one another (Satoh and Minami, 1995). Within the third intracellular loop is a binding site for the $G_{i/o}$ G-protein α subunits and of these, the $G\alpha_i$ is shown to inhibit the activity of adenylyl cyclase (Kurose *et al.*, 1983) and the $G\alpha_o$ subunit inhibits voltage-gated Ca^{2+} channels (Hescheler *et al.*, 1987), and with both G_i and G_o, activation of inwardly rectifying K^+ channels (Hescheler *et al.*, 1987). Overall, these effects result in reduced neuronal excitability through hyperpolarization which may explain the reduction in pain transmission associated with opioid use (Mansour *et al.*, 1995).

Underlying characteristics of the acute effects of opioids

Receptor Distribution

Distribution of opioid receptors throughout the periphery and central nervous system differs between subtypes and in part, underlie their observed pharmacological effects. Opioid receptors display a broad, but specific expression in many different tissues, including (but not limited to) the gastrointestinal tract, adrenal glands, kidneys, and reproductive organs (Wittert *et* *al.*, 1996). Their expression in the central nervous system varies but is widespread, with expression notable at both spinal and supraspinal levels. For the purpose for this dissertation, “supraspinal” is defined as a region above the spinal cord. Unsurprisingly, there is notable expression in brain regions mediating reward and motor function, such as the nucleus accumbens and striatum, and in regions dedicated to sensory processing, such as the thalamic nuclei (Tempel and Zukin, 1987; Mansour *et al.*, 1988). The analgesic effects of opioids are proposed to be mediated through a combination of spinal and supraspinal mechanisms (Figure 1.2). For instance, the periacqueductal grey (PAG), a region implicated in the analgesia elicited by opioids, displays high expression of MOR (Mansour *et al.*, 1988). Additionally, MOR expression can be seen in the dorsal and ventral horns of the spinal cord, as well as in the dorsal root ganglion. (Mansour *et al.*, 1988, 1995)

Opioid receptors, primarily the MOR in this context, are located within a pathway that serves to modulate incoming nociceptive information. This pathway is generally referred to as the descending modulatory pain pathway (Figure 1.2). Opioid receptors are one of many in a complex system that includes the likes of norepinephrine, serotonin, and dopamine. In terms of antinociception, this pathway functions in a manner to provide descending inhibition to reduce the excitability of primary afferent neurons (Millan, 2002).

Potency & Efficacy

Efforts to better understand the pharmacological profile of opioids has led to the development of additional opioid compounds, each varying in their affinities for opioid receptors and their efficacies at these receptors. Potency and efficacy are important components of a compound’s *in vivo* analgesic efficacy and are analyzed using a wide range of methodologies that range from *in vivo* characterizations with the whole animal to *in vitro* studies in cell homogenates.

Opioid compounds vary in their binding affinities for the MOR, KOR, and DOR. Due to the primary clinical use of MOR-targeted ligands in the treatment of pain, the remainder of this discussion will focus on MOR ligands (Pasternak and Pan, 2011). Affinity is defined as the "tenacity with which the drug binds to a receptor…it reflects the probability of the drug occupying the receptor at any instant in time" (Clarke and Bond, 1998) In some cases, a drug's binding affinity may serve as an indicator of a drug's relative potency. Potent drugs are capable of eliciting an effect by binding to some amount of receptor at low concentration by virtue of having high affinity for that receptor type. Less potent drugs which may have a lower affinity for a receptor require greater binding to elicit that same effect. The relative potency of MOR ligands are subject to variability across the system in which they are tested (i.e., in cell membranes, mouse vs. monkey tissue, etc.) and as a result, data sets can be inconsistent and sometimes incomplete.

In spite of the inconsistency among data sets, opioid agonists display a typical pattern of affinity and potencies. Typically, competitive binding studies to assess affinities using [^{3}H]-naloxone or [^{3}H]-DAMGO ([D-Ala^2, NMe-Phe^4, Gly-ol^5]-enkephalin). Both possess high affinities for the MOR and are used as a standard against which other opioid ligands can be compared (Pert *et al.*, 1973; Simon *et al.*, 1973; Handa *et al.*, 1981). Fentanyl and fentanyl-analogues and naloxone/naltrexone (opioid antagonists) are generally characterized as having the greatest affinities for the MOR (Emmerson *et al.*, 1994; Volpe *et al.*, 2011). The affinity of methadone for the MOR is controversial and has been reported as possessing both relatively high and relatively low affinity and in one case, a lower affinity agonist relative to morphine, but this may be related to differences in testing conditions (Chen *et al.*, 1991; Emmerson *et al.*, 1994; Volpe *et al.*, 2011). Similarly, the affinity of morphine is also dependent upon the conditions in which it is evaluated, where in some cases it demonstrates moderate affinity for the MOR but in others, its relative affinity is greater than that of fentanyl (Chen *et al.*, 1991; Volpe *et al.*, 2011). Oxycodone is generally ranked as having a lower relative affinity than morphine (Chen *et al.*, 1991; Volpe *et al.*, 2011).

It should be noted that although these compounds vary in their affinities for the MOR, poor in vitro binding does not necessarily preclude poor in vivo antinociceptive activity (Silvasti *et al.*, 1998; Volpe *et al.*, 2011). Agonist efficacy is defined as the capacity of a drug to activate a receptor and in this case, the capacity of an opioid ligand, such as morphine or oxycodone, to activate an opioid receptor (Clarke and Bond, 1998). Similar to variations in MOR affinity, opioid ligands also display an astounding variation in their efficacies. Opioid efficacy can be assessed using both in vivo and in vitro techniques but can vary as a function of behavioral or technical endpoints (i.e., the temperature of a noxious stimulus in vivo or drug incubation time in vitro) (Morgan and Christie, 2011).

Opioid efficacy in vivo can be assessed using a wide range of techniques, including the classical tail flick test (which utilizes a noxious thermal stimulus that can be adjusted for temperature intensity), the hot plate test, and many others. In vitro techniques utilize a direct approach of assessing MOR function as an indicator of opioid efficacy, and these include agonist-stimulated [^{35}S]GTPγS binding, receptor internalization studies, and studies of arrestin protein recruitment (Morgan and Christie, 2011). Most studies evaluate efficacy using agonist-stimulated [^{35S}]GTPγS binding in both cell culture models and native tissue. [^{35}S]GTPγS is an assay that was developed to evaluate the functional action of a drug and allows for rapid screening of compounds to determine if they are agonists, inverse agonists, or antagonists (Strange, 2010). The issue of differences between tissue and cell models still persists but overall the ranking of efficacy is similar, where DAMGO and methadone are among the highest efficacy agonists, followed by fentanyl and morphine being equi-efficacious, and then oxycodone as a lower efficacy agonist (Emmerson *et al.*, 1996; Selley *et al.*, 1997; Alt *et al.*, 1998). Based on earlier described efficacies in this paragraph, the opioid compounds can be ranked as such: DAMGO = Methadone > Fentanyl = Morphine > Oxycodone > Buprenorphine > Naltrexone (Table 1.1).

Determinants of opioid efficacy will inherently vary depending upon the endpoint but in the case of measuring maximal drug responses, there is the question of whether in vitro efficacy correlates with in vivo efficacy. As mentioned previously, there are a wide range of nociceptive tests that have been developed to assess the efficacy of opioid agonist. They vary in the types of stimuli used (thermal, chemical or mechanical), the duration of the pain state (acute vs chronic pain), and the subsequent behavior recorded (reflexive vs. supraspinally-organized behavior). The efficacy and potency of morphine to alter nociceptive responses varies as a function of the stimulus tested, whereby morphine is more efficacious in the tail withdrawal and hot plate tests but less efficacious in in the formalin test (Morgan *et al.*, 2006). In vivo determinations of opioid efficacy are subject to artificial constraints that serve to limit potential tissue damage to the animal (such as limiting stimulus exposure times) and for this reason, make it difficult to fully assess efficacy.

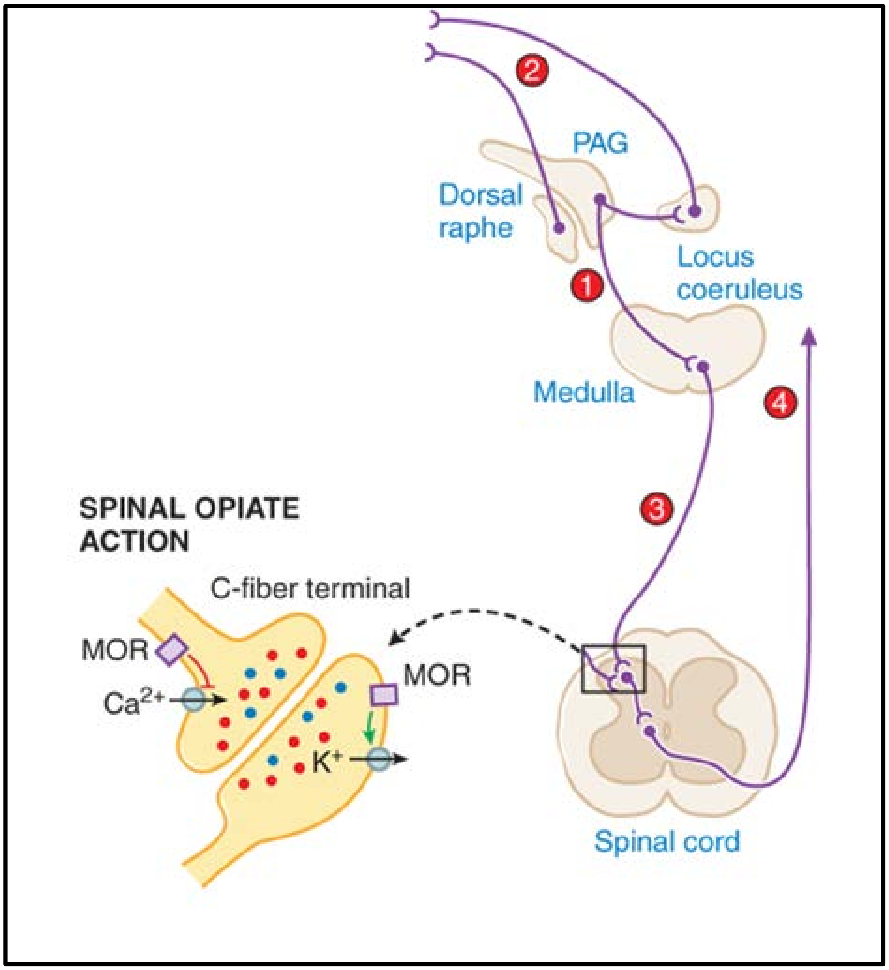

Figure 1.2: Distribution of mu-opioid receptors in the descending pain pathway. Mu-opioid receptors are distributed throughout regions that are important for the elicitation of opioid-induced antinociception. Neurons in the PAG project (1) to regions in the medulla, notably the rostral ventral medulla) and then projections from the medulla (3) directly modulate nociceptive afferents and interneurons in the dorsal horn of the spinal cord. The interneurons synapse on afferent neurons which then decussate and project back towards the brain and higher order structures (4). MOR is localized on primary afferent neurons in the dorsal horn of the spinal cord whereby it can directly modulate incoming nociceptive transmission. Opioids work by activating regions involved in a descending pain suppression mechanism in the spinal cord. Figure adapted from Goodman and Gilman's Manual of Pharmacology and Therapeutics, 2nd edition.

Opioid Ligand	**MOR Affinity**	**Relative Efficacy**
DAMGO	+++++ **	+++++
Buprenorphine	++++	+
Morphine	+++	++++
Fentanyl	+++	++++
Methadone	++	+++++
Oxycodone	+	++
Hydrocodone	+	++

Table 1.1: In vitro determinations of the relative affinity and efficacy of various MOR agonists. These approximations were derived from Volpe *et al.*, 2011. Agonist affinity was assessed using competition binding with [^{3}H]naloxone or [^{3}H]DAMGO. Agonist efficacy was determined using [^{35}S]GTPγS.**the affinity and efficacy of DAMGO were used as the reference for the relative affinity and efficacy of all opioid MOR agonists.

Tolerance

Acutely, the ability of opioids to alter the activity of descending pain pathway allows for their renowned antinociceptive/analgesic properties (Millan, 2002). In many cases, however, opioids are rarely administered just once, and in most cases, opioid treatment spans the course of days to weeks. Tolerance is a pharmacology/physiological adaptation that follows acute or repeated administrations of a drug such that increased doses of a drug are required to produce pharmacological effects that were previously elicited by smaller doses; this effect is characterized by a rightward shift of the dose-response curve (Savage *et al.*, 2003; Brunton *et al.*, 2011). The development of tolerance to the effects of opioids is not equivalent, as tolerance to the antinociceptive, euphoric, respiratory depressive, and constipating effects occur at different rates (Shook *et al.*, 1987; Ling *et al.*, 1989; White and Irvine, 1999; Ross *et al.*, 2008; Hill *et al.*, 2016).

For this reason, the diversity in opioid tolerance expression has led to its discussion as opioid *tolerances*. The differences in tolerances may be due to differences in their cellular effects. The extent to which these tolerances develop are dependent upon a multitude of factors: the dose of opioid, the frequency of administration, and the route of administration, to name a few (Paronis and Holtzman, 1992; Duttaroy and Yoburn, 1995; Fairbanks and Wilcox, 1997). Tolerance is a multifaceted phenomenon that encompasses changes in behavior, drug metabolism, receptor signaling, and changes in compensatory/inhibitory processes.

On a cellular level, opioid tolerance is thought to be regulated through the canonical GPCR mechanisms of desensitization, internalization, degradation, and downregulation (Figure 1.3) (Ferguson and Caron, 1998; Lefkowitz, 1998; Williams *et al.*, 2013). Desensitization refers to changes at the level of receptor signaling and is characterized as homologous or heterologous (where activation of one receptor leads to a convergence upon a signaling cascade and leads to desensitization of another receptor) (Stadel *et al.*, 1983; Sibley *et al.*, 1984, 1987; Hausdorff *et al.*, 1989). It's been suggested that desensitization is the acute loss of MOR-effector coupling and that this effect occurs within seconds to minutes after initial exposure to an opioid agonist (Kovoor *et al.*, 1998; Borgland *et al.*, 2003; Williams *et al.*, 2013).

Internalization is considered to be the recovery step from desensitization which occurs via endocytosis and leads to the eventual re-insertion of the resensitized receptor complex back into the plasma membrane (Ferguson *et al.*, 1996; Goodman *et al.*, 1996; Zhang *et al.*, 1996; Lefkowitz, 1998). Receptor internalization is ligand-specific and suggested to be dependent upon the intrinsic efficacy of the drug (Sternini *et al.*, 1996; Bohn *et al.*, 2004; McPherson *et al.*, 2010). High efficacy compounds, such as methadone, etorphine or DAMGO, rapidly induce MOR internalization following drug exposure, and relatively lower efficacy ligands, such as morphine, are less capable of inducing MOR receptor internalization (Keith *et al.*, 1996, 1998; Sternini *et al.*, 1996; Whistler and von Zastrow, 1998; Bohn *et al.*, 2004; McPherson *et al.*, 2010). Clearly, opioid agonists have substantial specificity in their ability to induce MOR internalization and it is of particular interest that morphine, a drug which possesses appreciable efficacy, is consistently reported to have impaired MOR trafficking.

Receptor downregulation refers to the reduction in overall availability of functional receptors that are present in the cell membrane (Williams *et al.*, 2013). Downregulation can be the result of increased receptor degradation following internalization or reduced biosynthesis of receptors (Law *et al.*, 1984, 1985; Klein *et al.*, 1986; Ronnekleiv *et al.*, 1996; Prenus *et al.*, 2012).

The rate and extent to which opioid tolerance develops can be altered by the addition of non-opioid ligands such as cannabinoids (Larson and Takemori, 1977; Trujillo and Akil, 1991; Smith *et al.*, 2007; Song *et al.*, 2015). In particular Δ^9-THC, have been investigated for the opioid-sparing properties and act in a synergistic manner with opioid co-administration in preclinical tests of antinociception (Welch and Stevens, 1992; Welch *et al.*, 1995). Several studies have shown that cannabinoids alter the development of acute tolerance to morphine, where co-administration of a low dose of THC with a low dose of morphine blocks MOR desensitization (Smith *et al.*, 2007). Cannabinoids are one such example of drugs that may alter the acute and chronic effects of opioids. The risks presented to patients taking opioids prompts a much-needed investigation into alternative means through which the pharmacological effects of opioids can be favorably altered. Therefore, in addition to altering the acute effects of an opioid with an adjunct that permits a lower dose needed to achieve analgesia, the rate and extent to which antinociceptive tolerance and dependence develop can also be thwarted as lower doses of opioid consumed are overall reduced.

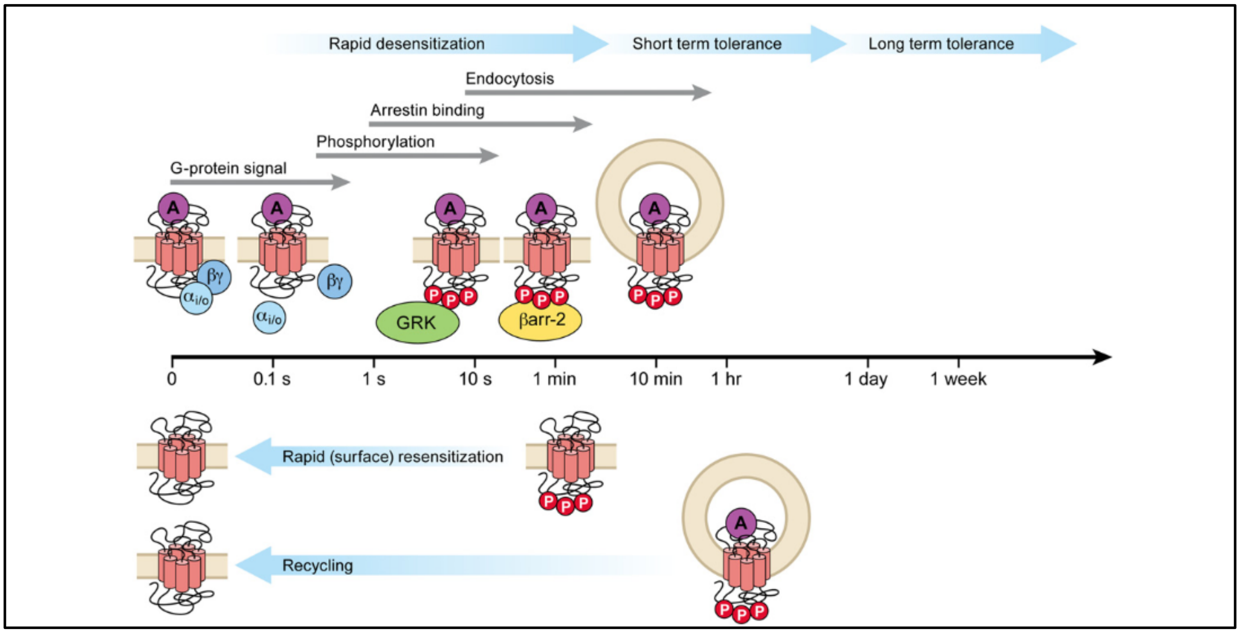

Figure 1.3: Time course of mu-opioid receptor trafficking following stimulation by an agonist. Upon binding of an agonist and initiation of G-protein mediated signaling, there is immediate recruitment of phosphorylating kinases, such as GRK, and subsequent binding of arrestin. Shortly after the desensitization process, endocytosis of the desensitized receptor occurs. The receptor can either undergo rapid re-sensitization or can be recycled. Short-term (acute) tolerance is defined as occurring within one day involves desensitization as a major process that precedes receptor endocytosis. Long-term, multiple-injection, tolerance is defined on a time scale of greater than one day and is presumed to require many compensatory mechanisms besides those described above.

(Williams *et al.*, 2013)

III. History of Serotonin Pharmacology

The history of serotonin is vast, with there being major bodies of literature detailing the role of serotonin in nearly every physiological function including but not limited to, mood, appetite, sleep, temperature regulation, gastrointestinal function and pain. Serotonin's functions and mechanisms are as diverse as its receptor subtypes (which will be explained in greater detail later) and its history is eventful. The discovery of serotonin and its receptors occurred during the golden age of receptor pharmacology, where the radioligand binding techniques were developed which allowed for the distinction of many different types of receptor types. In the words of Robert Lefkowitz, "if a single technical advance can be said to have opened the door to the molecular era of receptors, it was the development of radioligand binding methods during the 1970's" (Lefkowitz, 2004). Similar to the postulation of multiple types of opioid receptor, the existence of multiple serotonin receptors was hypothesized and subsequent subtypes later confirmed through radioligand studies.

The colorful history of serotonin as an endogenous neurotransmitter began far before the 1970s though, and in fact, as early at 1868 it was suspected that the blood contained a vasoconstrictive substance that would later be classified as serotonin (Richard Green, 2009). This substance was eventually characterized in the lab of Irvine Page where they were studying substances that were responsible for malignant hypertension (Rapport *et al.*, 1948). Eventually with the help of Arda Green and Maurice Rapport, the unknown substance was isolated from the serum component of two tons of coagulated bovine blood that was procured from a local slaughterhouse (Rapport *et al.*, 1948). They found that this compound was released from platelets during blood clotting and appropriately named it "serotonin" (or 5-hydroxytryptamine) because it was derived from serum and increased blood vessel tone (Rapport, 1948; Rapport *et al.*, 1948). In

1951, the synthesis of serotonin was confirmed and published by Hamlin and Fischer from Abbott laboratories (Hamlin and Fischer, 1951).

In the later 1930s, Vittorio Erspamer, a scientist in Rome, Italy, had discovered that secretions from enterochromaffin cells in the gastrointestinal tract contained a substance that produced intestinal contractions and uterine smooth muscle contractions (Erspamer and Boretti, 1951; Erspamer and Asero, 1952; Feldberg and Toh, 1953). This substance was dubbed "enteramine" as it had been isolated from the enteric nervous system and the compound's structure contained an indole ring. In 1953, serotonin and enteramine were reported to be identical compounds and shortly after, serotonin's presence in the brain was confirmed (Erspamer, 1952; Twarog and Page, 1953). Up to this point, serotonin was confirmed to be present in both peripheral tissues (the gut and platelets) and central tissues.

In 1957, the first two serotonin receptors were discovered in the guinea-pig ileum, named the "M" receptors (which can be blocked by morphine and thought to be in nervous tissue) and the "D" receptors (which are blocked by dibenzylaine and in muscle tissue) (Gaddum and Picarelli, 1957). For about twenty years after, serotonin-related discoveries slowed down but in 1979, there was a resurgence of interest in 5-HT receptor diversity. Peroutka and Synder (1979) demonstrated the presence of multiple serotonin binding sites using radiolabeled [^{3}H]5-Hydroxytryptamine, [^{3}H]LSD, and [^{3}H]Spiroperidol in frontal cerebral cortex and classified these distinct sites into two classes: 5-HT$_1$ and 5-HT$_2$. The 5-HT$_1$ class was further subdivided into 5-HT1$_A$, 5-HT$_{1B}$, and 5-HT$_{1C}$ (which would later be reclassified as 5-HT$_{2C}$) (Pedigo *et al.*, 1981; Palacios *et al.*, 2017).

Following the introduction of receptor cloning, many new serotonin receptors were identified and some reclassified (at that point, 5-HT$_{1C}$ became the 5-HT$_{2C}$) (Julius *et al.*, 1990). In 1994, a new classification scheme for serotonin receptors was introduced by Hoyer and up until that point, over 14 different 5-HT receptors were identified and all were GPCRs, except for 5-HT$_3$ (Hoyer *et al.*, 1994). See table 1.2 for the signaling pathways, expression, and function of the known 5-HT receptors.

Receptor Subtype	**Signal Transduction**	**Location**	**Function**
5-H$_{1A}$	↓ AC	Raphe nuclei, cortex, hippocampus)	Autoreceptors
5-HT$_{1B}$	↓ AC	Subiculum, globus pallidus, substantia nigra	Autoreceptor
5-HT$_{1D}$	↓ AC	Cranial vessels, globus pallidus, substantia nigra	Vasoconstriction
5-HT$_{1E}$	↓ AC	Cortex Striatum	----
5-HT$_{1F}$	↓ AC	Brain and periphery	----
5-HT$_{2A}$	↑PLC ↑PLA$_2$	Platelets, smooth muscle, cortex, spinal cord, PAG, striatum, cortex	Plate aggregation, contraction, neuronal excitation
5-HT$_{2B}$	↑PLC	Stomach fundus, kidneys, heart	Contraction
5-HT$_{2C}$	↑PLC ↑PLA$_2$	Choroid plexus, striatum, hippocampus, spinal cord, cortex, hypothalamus	CSF production, neuronal excitation
5-HT$_3$	Ligand-gated ion channel	Parasympthetic nerves, solitary tract, area postrema	Neuronal excitation
5-HT$_4$	↑AC	Hippocampus, GI Tract	Neuronal excitation
5-HT$_{5A}$	↓AC	Hippocampus	Unknown
5-HT$_6$	↑AC	Hippocampus, striatum, nucleus accumbens	Neuronal excitation
5-HT$_7$	↑AC	Hypothalamus, hippocampus, GI tract	Unknown

Table 1.2: Table of the serotonin receptor subtypes and their location and function. Table adapted from Goodman and Gilman's Manual of Pharmacology and Therapeutics, 2nd Edition (Hilal-Dandan and Brunton, 2016). Additional information cited from (Helton *et al.*, 1994; Choi and Maroteaux, 1996; Pierce *et al.*, 1996; López-Giménez *et al.*, 2001; Doly *et al.*, 2004).

IV. Serotonin in Pain Modulation

The role of serotonin in pain is established but the specific mechanisms through which serotonin may alter pain is unclear (for a review see Millan, 2002). Serotonin serves a dual role in both facilitating nociception and inhibiting nociceptive stimuli and this can be linked back to its diverse family of receptors and the sites at which these receptors are expressed (Hoyer *et al.*, 1994).

In the periphery, serotonin is a component of inflammatory responses but within the central nervous system (CNS), it plays dual roles in both nociceptive transmission and descending pain modulation (Tokunaga *et al.*, 1998; Bardin *et al.*, 2000; Jeong *et al.*, 2004; Kayser *et al.*, 2007; Nakajima *et al.*, 2008; Rahman *et al.*, 2011). For example, peripherally administered serotonin is reported to produce hyperalgesia by acting directly on nociceptors (Oliveira *et al.*, 2007). In cases of tissue injury, mast cells release serotonin that serves as an agent that produces both inflammation and potentiation of other inflammatory mediators (Taiwo and Levine, 1992; Hong and Abbott, 1994).

Serotonin is one of many components of an endogenous system that serves to modulate nociceptive transmission (Millan, 1997, 2002). Serotonergic cell bodies are localized in the raphe nuclei, and the projections of the serotonergic cell bodies innervate a vast majority of brain nuclei including the PAG or rostral ventral medulla (RVM) (Chan-Palay *et al.*, 1978; Yezierski *et al.*, 1982; Takeuchi *et al.*, 1983; Beitz *et al.*, 1986; Jones and Light, 1990; Zhang *et al.*, 2000) Neuronal projections from the PAG innervate the RVM and then project to the dorsal horns of the spinal cord (Castiglioi *et al.*, 1978; Yaksh and Tyce, 1979; Yaksh and Wilson, 1979; Aimone *et al.*, 1987; Cui *et al.*, 1999; Zhang *et al.*, 2000) Serotoninergic neurons only comprise ~20% of the neurons that project from the RVM to the dorsal horns, with the remainder being of non-serotonergic origin such as GABAergic (Ossipov *et al.*, 2010).

Early studies showed that stimulation of the PAG or RVM resulted in a release of serotonin from the spinal cord and intrathecal administration of serotonin was sufficient to produce antinociception (Yaksh and Wilson, 1979; Schul and Frenk, 1991). But the effect of spinal serotonin has the potential to be either inhibitory or faciliatory, with this effect depending upon the receptor subtype activated (Wilson *et al.*, 1979; Yaksh and Wilson, 1979; Bardin *et al.*, 2000; Jeong *et al.*, 2004). Although serotoninergic neurons only make up a small proportion of total neurons within the descending pain modulation system, it's the diverse receptor family that serves a critical role in modulating nociceptive transmission. For the sake of brevity, the remainder of this chapter will only focus on the 5-HT$_2$ receptor family but these receptors nonetheless exemplify this dual role of serotonin which will be expanded upon later.

The 5-HT$_2$ receptor class is comprised of three subtypes: 5-HT$_{2A}$, 5-HT$_{2B}$, and 5-HT$_{2C}$, and are G$_q$-coupled receptors which produce downstream effects through activation phosphoinsotide (PI) hydrolysis, increased Ca^{2+} mobilization, and inhibition of K$^+$ channel current conductance, which underlie their overall excitatory effect on neuronal activity (Boess and Martin, 1994). The receptors demonstrate a high level of sequence homology, where the 5-HT$_{2A}$ receptor shares an overall sequence identity of 53% with the 5-HT$_{2C}$ receptor and both the 5-HT$_{2A}$ and 5-HT$_{2C}$ receptors share an overall sequence identity of 43% with the 5-HT$_{2B}$ receptor (Julius *et al.*, 1990; Boess and Martin, 1994). Their conserved degree of sequence homology and functional activities, mainly similarities in signaling mechanisms (effect on PI metabolism) and pharmacological profiles, were the basis for their classification as members of the 5-HT$_2$ receptor family (Hoyer *et al.*, 1994).

It is of importance to note that the older literature has displayed a pattern of both pro- and anti-nociceptive roles for the 5-HT$_2$ receptors and for this reason, it was difficult to ascribe any particular pharmacological effects to any one receptor subtype (Rahman *et al.*, 2011). The development of increasingly selective agonists and antagonists, however, that can differentiate between subtypes has allowed for further characterization of the roles of each individual subtype (see Table 1.3).

5-HT$_{2A}$ Receptors

In recent years, multiple studies characterizing the role of peripheral and central 5-HT$_{2a}$ receptors in preclinical models of pain have been published (Abbott *et al.*, 1996; Tokunaga *et al.*, 1998; Millan, 2002; Okamoto *et al.*, 2002; Kayser *et al.*, 2007). Several lines of evidence suggest a direct role of serotonin in these nociceptive states which may be mediated through activation of the 5-HT$_{2A}$.

Peripheral 5-HT$_{2A}$ Receptors

The role of serotonin in peripheral nociception is hypothesized to be partially due to its direct effect on primary nociceptors in the peripheral tissues (Oliveira *et al.*, 2007). Immunohistochemical analysis of peripheral nerve fibers demonstrated anatomical localization of 5-HT$_{2A}$ receptors on unmyelinated sensory neurons in the dermal-epidermal junctions of glabrous skin and suggest that serotonin can produce its effect locally within the subcutaneous tissue (Carlton and Coggeshall, 1997). Under “normal” conditions (in the absence of a chronic pain or inflammatory pain state), these receptors are expressed on dorsal root ganglion neurons (DRGs), specifically on the small diameter C-fibers (Pierce *et al.*, 1996, 1997; Tokunaga *et al.*, 1998; Nicholson *et al.*, 2003). Several preclinical models show that inflammatory conditions induced by Conjugated Freund’s Adjuvant (CFA) or carrageenan results in an increased expression of 5-HT$_{2A}$ receptor mRNA in DRGs (Okamoto *et al.*, 2002; Liu *et al.*, 2005). This increase in DRG 5-HT$_{2A}$ receptor expression was also replicated in a model of peripheral neuropathy induced by the HIV

	K_i (nM)			
Compound	**5-HT$_{2A}$**	**5-HT$_{2B}$**	**5-HT$_{2C}$**	**Reference**
5-HT	21 ± 8^a	19 ± 5^a	2.4 ± 4^a	(Kimura *et al.*, 2004)
mCPP	16.1 ± 1^a	40 ± 9^a	16.1 ± 1^a	(Kimura *et al.*, 2004)
(-) DOI	1.1 ± 0.6	56.2 ± 5.3^b	4.8 ± 0.6	(Song *et al.*, 2005)
Ro 01075	24 ± 6^a	2.4 ± 0.1^a	19.2 ± 2^a	(Kimura *et al.*, 2004)
Lorcaserin	112	943^a	15	(Thomsen *et al.*, 2008)
Vabicaserin	3	152^a	14	(Dunlop *et al.*, 2011)
WAY 163909	212 ± 29	485 ± 49^a	10.5 ± 1.1	(Dunlop *et al.*, 2005)

Table 1.3: Competition binding affinity constants (K_i values) of 5-HT$_2$ receptor ligands for the Human 5-HT$_{2A}$, the human 5-HT$_{2B}$, and the human 5-HT$_{2C}$ receptors. Values listed in this table are the mean ± S.E.M. For the 5-HT$_{2A}$ and 5-HT$_{2C}$ receptors, K_i values were determined using [^{125}I]DOI except where indicated otherwise. ^{a} K_i values determined using [^{3}H]5-HT. ^{b} K_i values determined using [^{3}H]LSD.

medication, 2’,3’-dideoxycytidine (Van Steenwinckel *et al.*, 2009). These studies suggest that under healthy conditions, the 5-HT$_{2A}$ receptor serves a functional role in transmitting nociceptive information but in the case of pathological conditions, its expression pattern is altered and may contribute to the pathophysiology of neuropathic pain states.

Pharmacological studies utilizing ketanserin, a 5-HT$_2$ receptor antagonist that displays a preferential affinity for the 5-HT$_{2A}$ receptor, support a role of peripheral 5-HT$_{2A}$ receptors on sensory nociceptors. Intraplantar administration of ketanserin dose-dependently attenuates hyperalgesia induced by intraplantar 5-HT, using a measure of heat-stimulated paw withdrawal (Tokunaga *et al.*, 1998). A more specific evaluation of peripheral 5-HT$_{2A}$ receptors by Abbot (1996) demonstrated that intraplantar ketanserin dose-dependently attenuates the noxious effects of 5-HT and that administration of a selective 5-HT$_2$ receptor agonist (that exerts its primary effects through 5-HT$_{2A}$ receptors) produces a robust inflammatory state that is marked by nocifensive behaviors (licking, lifting, and favoring) (Abbott *et al.*, 1996). Though these studies provide compelling evidence for the purported 5-HT$_{2A}$ receptors, it’s worth noting that a major limitation of ketanserin is that in addition to antagonizing the 5-HT$_{2A}$ receptor, it also displays affinity for the 5-HT$_{2C}$ receptor.

Central 5-HT$_{2A}$ Receptors

The central nervous system is marked by a wide distribution of 5-HT$_{2A}$ receptors, including areas known to be involved in nociceptive processing (J F López-Giménez *et al.*, 1997; Juan F. López-Giménez *et al.*, 1997; López-Giménez *et al.*, 1998). Modulation of incoming nociceptive information occurs at multiple levels within the spinal cord and gross neuroanatomical characterization shows low to moderate expression of 5-HT$_{2A}$ receptors in the dorsal horn of healthy animals (Maeshima *et al.*, 1998; Zhang *et al.*, 2001). It is worth noting, however, that the motor neurons of the ventral horn show significant 5-HT_{2A} expression relative to the dorsal horn (Pompeiano *et al.*, 1994; Maeshima *et al.*, 1998; Doly *et al.*, 2004). The spinal cord neurons are also noted to display considerable localization on the post-synaptic plasma membrane (Doly *et al.*, 2004).

Similar to observations observed in the periphery, central 5-HT_{2A} receptors display sensitivity to pain states. For example, carrageenan-induced inflammation produces robust c-Fos (a marker of neuronal activation) immunoreactivity in the dorsal horn, with this effect antagonized by a local administration of ketanserin in the affected paw (Wei *et al.*, 2005). Further studies with carrageenan elucidated a distinct upregulation of 5-HT_{2A} receptor mRNA in the dorsal horn, also noting increased expression levels in the ventrolateral PAG grey and dorsal raphe nucleus (Zhang *et al.*, 2001). Peripheral neuropathy induced by administration of the HIV/AIDS therapy, 2',3'-dideoxycytidine, significantly increased 5-HT_{2A} receptor immunolabelling in the dorsal horn of mice relative to vehicle controls (Van Steenwinckel *et al.*, 2009). These data suggest a possible pro-nociceptive role of the 5-HT_{2A} receptor.

5-HT_{2B} Receptors

The functional role of the 5-HT_{2B} receptor has not been thoroughly characterized and its distribution remains controversial. The 5-HT_{2B} receptor has significant expression in the stomach fundus and mediates the smooth muscle contractile response induced by serotonin (Foguet *et al.*, 1992; Hoyer *et al.*, 1994). Immunohistochemical analysis confirms previous studies suggesting its expression in the gastrointestinal tract and, furthermore, was detected in both the myocardium and vascular endothelium (Choi and Maroteaux, 1996). Expression within the cardiac tissue is thought to underlie the potentially fatal valvopathy associated with activation of 5-HT_{2B} receptors and it has been recommended that all new drugs are to be screened against this receptor for activity (Rothman *et al.*, 2000).

The 5-HT_{2B} receptor displays modest CNS expression, with notable expression in discrete brain nuclei of the hypothalamus, amygdala, and septum (Duxon *et al.*, 1996). Expression of mRNA transcripts for 5-HT_{2B} receptor is found in the spinal cord but expression in dorsal root ganglion neurons remains controversial (Helton *et al.*, 1994). Wu et al. (2001) reported no 5-HT_{2B} receptor mRNA expression in DRGs and, in contrast, Nicholson et al. (2003) demonstrated mild expression of the 5-HT_{2B} receptor mRNA transcript, so there is no overall consensus. The 5-HT_{2B} receptor is implicated in the progression of peripheral neuropathy and an upregulation of mRNA 5-HT_{2B} receptor levels in the DRG are observed following chronic constriction injury (Urtikova *et al.*, 2012). This suggests a role of the 5-HT_{2B} receptor in the initiation and maintenance sustained pain states and may be another mechanism through which interventions can be developed.

The 5-HT_{2B} receptor is implicated in the pathophysiology of serotonin-induced mechanical hypersensitivity but this effect is confounded by the use of an antagonist that possess appreciable affinity for both the 5-HT_{2B} and the 5-HT_{2C} receptor (Lin *et al.*, 2011). In support of this idea, further study with an antagonist (that possesses greater selectivity for the 5-HT_{2B} receptor) attenuated visceral hypersensitivity induced by 2,4,6-trinitrobenzene sulphonic acid (TNBS) and restraint stress (Ohashi-Doi *et al.*, 2010). Although the functional significance of the 5-HT_{2B} receptor in pain is debated, early data suggests a role in the modulation of nociceptive processing that should be evaluated following further development of more selective ligands.

5-HT_{2C} Receptors

The 5-HT_{2C} receptor is a G-protein-coupled-receptor that signals through the G_q pathway and is the only known GPCR that undergoes post-transcriptional mRNA editing to yield diverse receptor isoforms (Fitzgerald *et al.*, 1999). Its expression is considered to be restricted to the central nervous system with little basal expression observed in the periphery. In the CNS, 5-HT$_{2C}$ receptor expression is observed in several regions related to nociception, including the dorsal and ventral horns of the spinal cord and the thalamus (Pompeiano *et al.*, 1994). The role of 5-HT$_{2C}$ receptors in nociception, however, is in some ways unclear due to the previous lack of available selective agonists that would incidentally signal through the 5-HT$_{2A}$ or 5-HT$_{2B}$ receptors (Serafine *et al.*, 2015). With the recent development of selective agonists, such as lorcaserin and vabicaserin, additional studies can be conducted to further investigate the role of the 5-HT$_{2C}$ receptor in nociception and pain (Thomsen *et al.*, 2008; Dunlop *et al.*, 2011).

Several lines of evidence point to the involvement of the 5-HT$_{2C}$ in nociception. First, the receptor is expressed within the dorsal and ventral horns of the spinal cord and is optimally placed to modulate nociceptive afferents in the superficial and deeper lamina (Fonseca *et al.*, 2001). Secondly, the 5-HT$_{2C}$ receptor is also expressed in the thalamus, the critical relay station for all ascending sensory tracts before synapsing in the cortex (Clemett, *et al.*, 2000).

The role of the 5-HT$_{2C}$ receptor in peripheral inflammation and pain is heavily debated, as the current literature suggests that 5-HT$_{2C}$ receptor expression is limited to the CNS (Julius *et al.*, 1988; Clemett, *et al.*, 2000; López-Giménez *et al.*, 2001). Recent evidence suggests however that its peripheral expression may be dependent upon a pathophysiological state. Under normal physiological conditions, there is little expression of 5-HT$_{2C}$ receptor mRNA in DRGs but after treatment with CFA, DRGs show a marked induction of 5-HT$_{2C}$ receptor mRNA expression (Pierce *et al.*, 1996; Nicholson *et al.*, 2003). A similar induction of 5-HT$_{2C}$ receptor mRNA is also observed after an injection of bee venom into the hind paw of rats (Liu *et al.*, 2005). These data suggest that the 5-HT$_{2C}$ receptor may underlie the some of the pathophysiological adaptations that occur following the induction of chronic pain states but the mechanism through which it is acting has yet to be elucidated.

Another interesting piece of evidence is that intraplantar administration of selective 5-HT$_{2C}$ receptor antagonists, SB242084 and RS-10221, attenuates formalin-induced paw-withdrawal behavior and reduces C-Fos expression in the superficial laminae of the dorsal horn in rodents (Nakajima *et al.*, 2008). Unlike the forthcoming studies, this is one the first experiments to suggest the existence and a possible role of peripheral 5-HT$_{2C}$ receptors in the elicitation of nociception.

In most studies, 5-HT$_{2C}$ receptor agonists are administered via the intrathecal route and it is unclear why these agonists are typically inactive when administered systemically (Obata *et al.*, 2004, 2007; Nakai *et al.*, 2010). Administration of intrathecal 5-HT$_{2C}$ receptor agonists – MK212, Ro 60-0175 or WAY-161503, produced a dose-dependent attenuation of mechanical hypersensitivity induced by a chronic constriction injury in rodents (Nakai *et al.*, 2010). Consistent with this finding, intrathecal administration of another 5-HT$_{2C}$ receptor agonist produces antiallodynic effects in a rodent model of peripheral neuropathy (Obata *et al.*, 2007). Curiously, the antiallodynic effects of these agonists were attenuated by administration of muscarinic and α_2-adrenergic antagonists, suggesting that these receptor systems may partially mediate the antinociceptive effects of 5-HT$_{2C}$ receptor agonists.

Although 5-HT$_{2C}$ receptor agonists as antinociceptive agents are administered via the intrathecal route, it should be noted that systemic administration of antinociceptive 5-HT$_{2C}$ agonists has been reported (Ogino *et al.*, 2013). 5-HT$_{2C}$ receptor agonists, including lorcaserin and vabicaserin, display antinociceptive effects when administered systemically in a preclinical model of fibromyalgia (Ogino *et al.*, 2013) Fibromyalgia is a musculoskeletal disorder that is characterized by chronic pain and can be modeled in rodents by treating animals with reserpine (Ogino *et al.*, 2013).

V. Opioids & 5-HT2c agonists

Early studies from the 1970s demonstrated that serotonergic signaling is an important component of opioid analgesia. Though opioid analgesia is primarily mediated through mu opioid receptor (MOR) activation, descending serotonergic spinal projections were discovered as an important component (Yaksh and Tyce, 1979; Aimone *et al.*, 1987; Paul *et al.*, 1988; Schul and Frenk, 1991; Cui *et al.*, 1999). This descending input originates from the periaqueductal grey (PAG), synapses in the rostral ventral medulla (RVM), before finally projecting downward into both the contralateral and ipsilateral dorsal and ventral horns, where it modulates incoming nociceptive afferents and outgoing motor efferents (Millan, 2002).

The necessity of serotonin in the elicitation of morphine analgesia is supported by the observation that 1) depletion of serotonin by pharmacological inhibition of synthetic enzymes reduced morphine efficacy (Tenen, 1968); 2) intrathecal administration of serotonin antagonists attenuated morphine-induced antinociception (Wigdor and Wilcox, 1987; Paul *et al.*, 1988); 3) morphine administration evoked the release of spinal serotonin (Yaksh and Tyce, 1979; Tao *et al.*, 2002); 4) morphine increased serotonin metabolic turnover (Raffaello *et al.*, 1975; Theiss *et al.*, 1975); and 5) administration of selective-serotonin reuptake inhibitors (SSRIs) or tricyclic antidepressants (TCAs) enhanced morphine's antinociceptive effects (Larson and Takemori, 1977; Kellstein *et al.*, 1984; Hynes *et al.*, 1985; Banks *et al.*, 2010; Li *et al.*, 2011). Serotonin and opioid systems work in a cooperative fashion and all components may be necessary to achieve full expression of opioid-induced antinociception (Dewey *et al.*, 1970; Crisp *et al.*, 1991; Cui *et al.*, 1999; Li *et al.*, 2001; Lo *et al.*, 2004; Aira *et al.*, 2012).

The role of a 5-HT$_{2C}$ receptor agonist, like lorcaserin, in the elicitation of opioid-induced antinociception is even less clear. In the past few years, however, two studies have emerged demonstrating 5-HT$_{2C}$ agonists as potential treatments for opioid dependence. Lorcaserin, a 5-HT$_{2C}$ agonist, and reported to attenuate naloxone-precipitated withdrawal in animals that are chronically administered either morphine or heroin (Wu *et al.*, 2015; Zhang *et al.*, 2015). In addition, chronic administration of morphine increased 5-HT$_{2C}$ receptor expression in the nucleus accumbens, locus coeruleus, and ventral tegmental area (Wu *et al.*, 2015; Zhang *et al.*, 2015).

Currently, lorcaserin (in combination with extended release naltrexone) is undergoing clinical trial testing for the treatment of opioid use disorder (OUD) (ClinicalTrials.gov, 2017). In addition to understanding how lorcaserin alters OUD, it is important to understand the effect of lorcaserin on opioid antinociception.

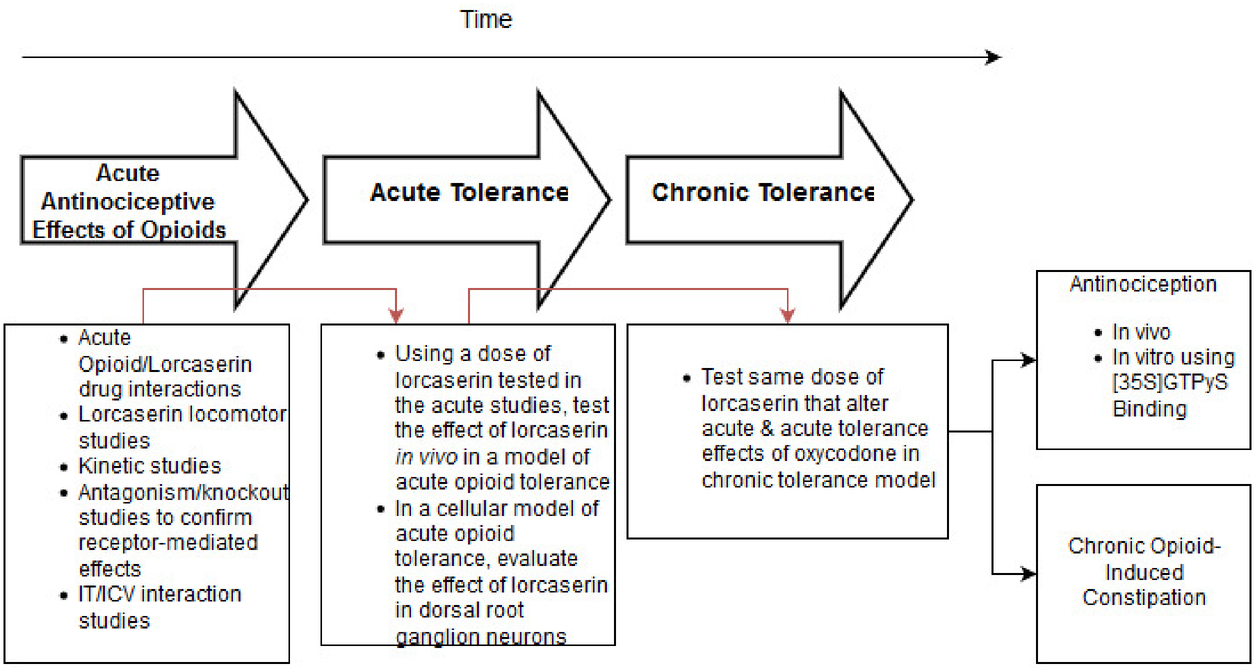

Figure 1.4: Experiments planned for the dissertation and how they relate to one another. The initial studies evaluated the acute interactions between lorcaserin and opioids, primarily oxycodone. From these studies, we used a dose of lorcaserin (2 mg/kg, s.c.) that significantly shifted the oxycodone dose-response curve to evaluate lorcaserin's effects on acute chronic tolerance. Acute tolerance is often thought to be the initial stage preceding chronic tolerance, therefore, it was important to evaluate lorcaserin's effects in this stage first using *in vivo* and *in vitro* approaches. The development of "chronic tolerance" follows the development of acute tolerance. The same dose of lorcaserin (2 mg/kg, s.c.) that altered the acute interactions and acute tolerance to oxycodone was tested in the models of chronic tolerance in models of antinociception and opioid-induced constipation. These studies provide insight into the temporal effect of lorcaserin on opioid pharmacology.

VI. Models to Investigate Interactions

Investigations of the acute and chronic interactions of lorcaserin and oxycodone are important if lorcaserin will ever be translated into a possible opioid-sparing therapy in the clinic. Figure 1.4 provides a visual schematic of the relationship between the experiments and how they relate to one another. Acute interactions were evaluated using the warm-water tail-withdrawal assay, which is used as a model of acute pain. The tail-withdrawal assay has been used repeatedly and shown to be an adequate predictor of opioid-mediated analgesic effects and has been used to evaluate drug-drug interactions for other opioid-sparing compounds (Welch and Stevens, 1992; Fairbanks and Wilcox, 1999; Raffa *et al.*, 2000; Cichewicz and McCarthy, 2003; Smith *et al.*, 2007; Williams *et al.*, 2008; Stone *et al.*, 2014).

Using the tail-withdrawal procedure, the acute interactions were evaluated as follows. First, the dose-relationship effect of lorcaserin alone was evaluated because it is important to evaluate the effect of each drug on its own prior to combination testing. In addition to this, the effect of lorcaserin on locomotor activity was evaluated because general behavioral sedation is a potential confound that may affect the perceived antinociceptive properties of a drug (Negus *et al.*, 2006) Second, the dose-related and time-course of lorcaserin's effects on opioid antinociception were characterized. Following this analysis, studies evaluating the contributions of the 5-HT$_{2A}$ receptor (through use of a knockout model) and the 5-HT$_{2C}$ receptor (using the selective 5-HT$_{2C}$ receptor antagonist SB242084) were conducted because lorcaserin has notable activity at both of these receptors (Thomsen *et al.*, 2008). In addition to evaluating the pharmacodynamic interactions of lorcaserin and oxycodone, studies were conducted to evaluate the effect of lorcaserin on the biodistribution of oxycodone. Lorcaserin is reported as a competitive inhibitor of the CYP2D6 enzyme and it is important to evaluate any potential changes in opioid metabolism that may be underlying the observed antinociceptive interactive effects (Center for Drug Evaluation and Research, 2012).

In order to fully characterize the pharmacology of lorcaserin, it's effects were evaluated via intrathecal and intracerebroventricular routes of administration. Previous opioid-sparing adjunctive therapies, such as clonidine, Δ^9-THC, and acetaminophen, have been evaluated via i.t. and i.c.v. routes of administration (Ossipov *et al.*, 1985, 1988; Lichtman and Martin, 1991; Welch and Stevens, 1992; Fairbanks and Wilcox, 1999; Raffa *et al.*, 2000; Stone *et al.*, 2014). Although these studies do not directly provide insight into the opioid-sparing potential of lorcaserin, they provide a general anatomical locus of activity and insight into the role of 5-HT$_{2C}$ receptors in nociception.

Following characterization of the acute interactions between lorcaserin and oxycodone, the effect of lorcaserin on the effect of repeated oxycodone administration was evaluated. Tolerance is thought to be comprised of two components: an acute (short-term) component and a chronic (long-term) component (Cox et al., 1968; Rosenfeld et al., 1977; Huidobro-Toro and Way, 1978; Fairbanks and Wilcox, 1997; Bohn et al., 2000; Williams et al., 2013). In the acute tolerance studies, the effect of lorcaserin was evaluated *in vivo* using a dosing paradigm that has been extensively validated in the literature (Cox et al., 1968; Huidobro-Toro and Way, 1978; Ling et al., 1989; Fairbanks and Wilcox, 1997; Bohn et al., 2000). Further studies were conducted to evaluate the effect of lorcaserin on acute opioid tolerance at a single-cell level in dorsal root ganglion neurons. After characterization of lorcaserin's effects in the models of acute tolerance, the effect of lorcaserin to alter the development of chronic opioid tolerance *in vivo* was assessed and then based upon the results generated from that study, further tests were conducted using a measure of MOR-mediated functional activity. Collectively, these studies provide insight into the potential of lorcaserin as an opioid-sparing adjunct and the possible mechanisms through which lorcaserin maybe working.

In vivo models

Rationale for mouse sex and strains tested.

In order to complete a thorough pharmacological evaluation of the effect of lorcaserin on oxycodone antinociception, male mice were exclusively tested in all paradigms. Previous data on lorcaserin were generated in primarily male subjects and this project aimed to be consistent with the literature (Higgins *et al.*, 2012; Ogino *et al.*, 2013; Wu *et al.*, 2015; Zhang *et al.*, 2015; Banks and Negus, 2016; Harvey-Lewis *et al.*, 2016; Neelakantan *et al.*, 2017). Future studies should evaluate the effect of lorcaserin on opioid antinociception in female subjects because significant differences in serotonin synthesis, serotonin receptor expression and distribution, and serotonin transporters are reported (Carlsson and Carlsson, 1988; Nishizawa *et al.*, 1997; Zhang *et al.*, 1999; Cannon *et al.*, 2013).

The primary mouse strain used in these studies was the swiss webster (SW) outbred mouse from Envigo (Frederick, MD). The SW mice are routinely used in this laboratory to characterize the antinociceptive activity and tolerance of opioid compounds, and to be consistent, they were used in these studies for appropriate comparison to previous data generated from our lab. In the electrophysiology studies, C57/B6J mice were purchased from Envigo (Frederick, MD). C57/B6J mice were used because previous testing in our lab has demonstrated that the dorsal root ganglion neurons from the SW mice are difficult to patch on and maintain a strong seal to record from within the neuron. The 5-HT_{2A} receptor knockout studies were generated on a 129Sv background and were the only strain of mouse available for these studies.

Doses of lorcaserin tested for studies. Doses of lorcaserin and oxycodone were generated using a Log_2 scale (0.25, 0.5, 1, 2, 4, 8….). Lorcaserin has previously been tested at doses on the log scale and range as used in these studies from 0.125, 0.25, 0.5, and 1 mg/kg, and these studies also primarily utilized subcutaneous administration of lorcaserin (Levin *et al.*, 2011; Wu *et al.*, 2015; Zhang *et al.*, 2015; Neelakantan *et al.*, 2017). Lorcaserin was administered subcutaneously in the initial studies based on the work of the previously cited work.

Although this is not necessarily a clinically relevant route of administration for lorcaserin, we felt that the studies should be comparable to previously published research. A few years into the generation of the work described herein, I found a paper that described the effect of lorcaserin administered orally, and that study demonstrated the oral efficacy of lorcaserin to attenuate mechanical hypersensitivity in a preclinical chronic pain model of fibromyalgia (Ogino *et al.*, 2013). Although these studies evaluated lorcaserin subcutaneously, future studies should investigate the interactions between oral oxycodone and oral lorcaserin. In general, most studies that evaluate the opioid-sparing effects of a novel compound administer it via the subcutaneous route of administration and it is only when results are encouraging that additional testing is conducted using the clinically relevant route (in this case, p.o.).

Warm-Water Tail Withdrawal

The warm-water tail-withdrawal test utilizes a thermal stimulus that stimulates thermoreceptors and nociceptors in the skin. The test is a modified version of the tail-flick test using radiant heat by D’Amour and Smith (1941) and was developed as a simplified, preclinical version of the method tested on human subjects by Hardy et al. (1940). In practice, a thermal stimulus (heated water) is applied to the distal end of a rodent’s tail which provokes a withdrawal response by way of a vigorous movement. Reaction time of this withdrawal is recorded and referred to as the "latency to withdraw" or "tail-flick latency." An artificial cut-off threshold of typically 10 seconds is imposed to prevent the incidence of tissue damage, otherwise skin burning may occur.

The tail-withdrawal is demonstrated to be primarily a spinally-mediated reflex because the response persists even after resection of upper components of the spinal cord (Irwin *et al.*, 1951). The tail withdrawal reflex is also subject to modulation by supraspinal structures as stimulation of the tail resulted in recorded neuronal activity in the thalamus and additionally, reports of increased flexor reflex following spinal resection suggest that supraspinal structures may provide inhibitory tone (Irwin *et al.*, 1951; Mitchell and Hellon, 1977).

Opioids in this paradigm work by inhibiting the withdrawal response and this effect is a combination of spinal and supraspinal mechanisms (Dewey *et al.*, 1969; Wu and Martin, 1982; Bell *et al.*, 1985; Sinclair *et al.*, 1988). Decerebration of the spinal cord reduces the potency of morphine and suggests that the antinociceptive effects of morphine are dependent on both spinal and supraspinal structures (Dewey *et al.*, 1969; Wu and Martin, 1982).

Opioid analgesics have been shown to significantly inhibit this reflexive tail-withdrawal response and reliably do so across many species (Dykstra and Woods, 1986; Le Bars *et al.*, 2001). The tail flick/tail withdrawal assay is particularly sensitive to the antinociceptive opioid agonists including MOR and KOR agonists, but the assay is insensitive and lacks predictive validity to determine to the antinociceptive effects of systemically-administered non-opioid analgesics such as NSAIDs (Negus *et al.*, 2006; Dogrul *et al.*, 2007; Foroud and Vesal, 2015). Although the tail withdrawal assay has its limitations, this test has been shown to be predictive of analgesic effects mediated through the MOR in human populations and has remained a mainstay for testing the analgesic potential of new opioid compounds (Le Bars *et al.*, 2001).

Locomotor Studies

Most assays that are used to assess nociception (and subsequently antinociception) rely on animals to engage in motor responses to noxious stimuli. A potential confound of testing candidate analgesic drugs are drug effects that produce motor impairment and general behavioral depression (Le Bars *et al.*, 2001; Negus *et al.*, 2006). An “antinociceptive” response may be confounded by a subject’s impaired ability to respond to a noxious stimulus and may be interpreted as a false positive result. Tests such as the warm-water tail withdrawal are intrinsically dependent upon the elicitation of motor responses and mark stimulus sensitivity thresholds which may be manipulated by the addition of an antinociceptive compound. This is a known and appreciated limitation of assays like the warm-water tail withdrawal test. The assessment of locomotor activity provides some insight to ensure that the observed antinociceptive effect is not due to motor impairment blocking the nocifensive behaviors.

5-HT$_{2A}$ Knockout Mice

A common issue among commercially available 5-HT$_2$ receptor agonists (until recently) is their indiscriminate activity at all 5-HT$_2$ receptors. Previously tested compounds, such as DOI (2,5-Dimethoxy-4-iodoamphetamine), Ro 60-0175, or mCPP [1-(3-Chlorophenyl)piperazine)], possess affinity for the 5-HT$_{2C}$ receptor but additionally display varied affinity and efficacy at the 5-HT$_{2A}$ and 5-HT$_{2B}$ receptors (Porter *et al.*, 1999; Kimura *et al.*, 2004; Cheng and Kozikowski, 2015). Lorcaserin was one of the first agonists that displayed preferred activity at the 5-HT$_{2C}$ receptor and demonstrated greater selectivity over the 5-HT$_{2A}$ and the 5-HT$_{2B}$ receptors (Thomsen *et al.*, 2008). Although lorcaserin has greater selectivity, the possibility of off-target effects, likely mediated through the 5-HT$_{2A}$ receptor, were of concern. As a means to assess contributions of the 5-HT$_{2A}$ receptor in the effect of lorcaserin on opioid antinociception, a global knockout model of the 5-HT$_{2A}$ receptor was used to assess the effect of 5-HT$_{2A}$ receptor deletion on the combined effects of lorcaserin and opioids. Although as previously mentioned early in this chapter, the 5-HT$_{2A}$ receptor primarily serves a “pro-nociceptive” role, it was important to rule out its contributions.

Biodistribution Studies

Drug-drug interactions are a major clinical concern, as alterations in drug concentrations may have fatal effects on a patient. Opioid drugs differ in the mechanisms through which they are metabolized and there is great variability in metabolic pathways among patient populations (Poyhia ’ *et al.*, 1992; Stamer *et al.*, 2013). Opioids, such as oxycodone and fentanyl, are subject to first pass hepatic effect and are subsequently metabolized by CYP3A4 and to a lesser extent CYP2D6 (Smith, 2009; Söderberg Löfdal *et al.*, 2013). Though each opioid may vary in enzyme metabolism, there is potential for interactions with other drugs that may act as substrates, inhibitors, or inducers of those enzymes. The net effect of these effects may be increased circulating opioid concentrations, which presents itself as an increased analgesic effect and increased risk of adverse side effects such as respiratory depression. There are many agents that may alter enzyme function but a few examples include antibiotics, SSRIs, and some antipsychotics (Crewe *et al.*, 1992; Ball *et al.*, 1997; Chiu *et al.*, 2004; Smith, 2009).

Due to the potential for substantial drug-drug interactions between opioids and lorcaserin, it was important to evaluate the bio-disposition of oxycodone with and without lorcaserin pretreatment. Lorcaserin is similarly subject to metabolism by CYP P450 enzymes and is a competitive inhibitor of CYP2D6 (which is responsible for some oxycodone metabolism) (Samer *et al.*, 2010; Center for Drug Evaluation and Research, 2012). Understanding the effect of lorcaserin on opioid biodisposition is an important step in characterizing the effect of lorcaserin on opioid antinociception and ensuring that the effects observed *in vivo* are not mediated through (potentially fatal) changes in opioid metabolism.

In vivo Models of Tolerance

Tolerance is defined as the reduction in response to a drug after repeated administration and is expressed as a right-ward shift of the dose response curve (Brunton *et al.*, 2011). Tolerance to the antinociceptive effects of opioids can be modeled using a variety of dosing paradigms that range from a single injection (acute tolerance) to multiple injections on the time-scale of a few days or a few weeks of treatment. The mechanisms that underlie the degree of tolerance that develops differ based on frequency with which the opioid is administered, and the induction of antinociceptive tolerance is also considered to occur in two phases: an acute component and a chronic state (Cox et al., 1968; Huidobro-Toro and Way, 1978; Ling et al., 1989; Fairbanks and Wilcox, 1997; Bohn et al., 2000, Tempel *et al.*, 1988; Z Wang *et al.*, 1994; Sim *et al.*, 1996; Sim-Selley, 2005). Indeed, this idea has been supported by several lines of research demonstrating that agents that alter morphine tolerance do not equivalently alter acute and chronic tolerance (Rosenfeld and Burks, 1977; Fairbanks and Wilcox, 1999). activity

The injection method that we are using to test acute tolerance in these studies is based on a model developed by Cox *et al.*, (1968) and later adapted by Wigdor and Wilcox (1987). The time frame of the drug treatment occurs within a day and may be limited to a single drug administration in a day or repeated drug exposures within a day. The mechanisms that underlie acute tolerance are considered to be more well-understood and mediated through rapid receptor desensitization that results in an acute loss of MOR-effector coupling (Sibley *et al.*, 1984, 1985, 1987; Ferguson *et al.*, 1996; Kovoor *et al.*, 1998; Laura M. Bohn *et al.*, 2000; Alvarez *et al.*, 2002; Bailey *et al.*, 2004; Williams *et al.*, 2013; Arttamangkul *et al.*, 2018).

The degree of tolerance that develops following multiple opioid exposures across a period of several days (long-term tolerance) is thought to be mediated through mechanisms that are distinct from those responsible for acute tolerance (Tempel and Zukin, 1987; Tempel, 1991; Tao *et al.*, 1993; Z Wang *et al.*, 1994; Wang *et al.*, 2004; Sim-Selley, 2005; Shoblock and Maidment, 2006; Sim-Selley *et al.*, 2009). It is marked by compensatory changes in regulatory processes and receptor downregulation. The model of chronic tolerance varies by several factors including, but not limited to, the route of administration (systemic vs spinal), the dose and dosing frequency of opioid administered (acute vs. chronic dosing), the species of the animal (rodent vs monkey), and the method through which tolerance will be evaluated (tail flick vs. hot plate). Though these factors vary, the general consensus is that "chronic" tolerance is a series of multiple injections across multiple days (Fairbanks and Wilcox, 1997; Williams *et al.*, 2013). The model of long-term tolerance used in these studies has been previously published and produces profound, reproducible antinociceptive tolerance to oxycodone (Jacob *et al.*, 2017).

Both the acute and multiple-dosing/chronic models of tolerance are useful because they provide an approximate framework through which the effect of an additional drug, in this case lorcaserin, can be evaluated. It is well-known that the addition of non-opioid compounds, such as NMDA antagonists, differentially alter the acute and chronic phases of tolerance (Trujillo and Akil, 1991; Pasternak *et al.*, 1995). Acute tolerance in the clinic is a debated phenomenon and studies report that acute tolerance may develop following intraoperative administration of remifentanil and this treatment increases post-operative opioid consumption (Vinik and Kissin, 1998; Schraag *et al.*, 1999; Guignard *et al.*, 2000; Cortínez *et al.*, 2001; Gustorff *et al.*, 2002; Dworkin *et al.*, 2007). Though acute tolerance in the clinical setting is debated, it is clearly an important component worth investigating as it provides insight in the overall mechanisms that opioid tolerance can be modulated.

In vitro models

Electrophysiology

As mentioned previously, tolerance is characterized by a loss of response to a drug treatment. Electrophysiological measures of neuronal activity are used as a reliable measure that are altered by repeated drug exposures. The hallmark effects of opioids on neurons include an increase in threshold potential and a reduction in action potential amplitude. Tolerance to the effect of opioids can be evaluated through measures of neuronal excitability and is a reproducible model to evaluate tolerance on a neuronal level (Kang *et al.*, 2017; Jacob *et al.*, 2018). Dorsal root ganglion neurons (DRGs) are a model used to evaluate the development of tolerance at this level because of their critical role as a “relay station” between peripheral nociceptors/stimuli and the central nervous system.

DRGs express a wide-variety of receptors, including MORs and a variety of serotonin receptors (Pierce *et al.*, 1997; Nicholson *et al.*, 2003). Expression of the 5-HT$_{2C}$ receptor in DRGs is debated, with the caveat being that their basal expression is in such low quantities that it is difficult to detect via PCR analysis or via radioligand competition binding (Pierce *et al.*, 1996, 1997; Chen *et al.*, 1998; Nicholson *et al.*, 2003). The 5-HT$_{2C}$ receptor has been implicated in the initial stages of neuronal sensitization following the induction of a chronic pain state, as it displays an upregulation of mRNA following injury with CFA or bee venom (Wu *et al.*, 2001; Liu *et al.*, 2005).

The DRGs are an ideal target for evaluating opioid tolerance and its modulation by activation of the 5-HT$_{2C}$ receptor for several reasons. Although tolerance is primarily thought of as a centrally-mediated phenomenon, maladaptive changes in peripheral nociceptors are implicated as the initial site for the development of analgesic tolerance (Corder *et al.*, 2017). Opioid tolerance within the afferent cell bodies has been repeatedly demonstrated and is a well-regarded phenomenon (Kang *et al.*, 2017; Jacob *et al.*, 2018). Although the expression and role of the 5-HT$_{2C}$ receptor is debated, electrophysiological methods using the DRGs allow for a functional, though indirect, assessment of the role of the 5-HT$_{2C}$ receptor on opioid tolerance. Opioid tolerance can be modulated through administration of several exogenous compounds, including ethanol and a protein kinase C inhibitor (Bailey *et al.*, 2004; Hull *et al.*, 2010; Jacob *et al.*, 2018). Use of this methodology will allow for the characterization of lorcaserin's effects on a single cell level (in a cell type that serves a critical role in nociception and opioid tolerance) and provide an understanding of how it relates to observations *in vivo.*

[^{35}S] GTPγS Binding

The mechanisms of opioid tolerance are expressed in many levels of an organism, including at the receptor level. Tolerance at this level is marked by a loss of MOR-effector coupling through desensitization and an overall receptor downregulation (Tempel *et al.*, 1988; Tempel, 1991; Ronnekleiv *et al.*, 1996; Kovoor *et al.*, 1998; Whistler and von Zastrow, 1998; Alvarez *et al.*, 2002; Borgland *et al.*, 2003; Lopez-Gimenez *et al.*, 2008). The [^{35}S]GTPγS binding assay is a measure of receptor mediated G-protein activation that can be altered through the addition of opioid agonists and antagonists (Selley *et al.*, 1997). It is useful in applications of analyzing acute opioid efficacy and opioid tolerance. The loss of MOR-effector coupling is proposed as one such mechanism that may underlie tolerance and [^{35}S]GTPγS is an appropriate tool to examine changes in first stage of initial receptor-mediated signaling following chronic opioid exposure (Celver *et al.*, 2004).

Region-specific decreases in MOR-uncoupling or “desensitization” are reported in the spinal cord, PAG, and pontine and medullary nuclei following chronic *in vivo* opioid exposure (Tao *et al.*, 1993; Sim *et al.*, 1996; Sim-Selley *et al.*, 2009). Combination treatments utilizing an opioid and a non-opioid compound, in this case Δ^9-THC, were shown to not produce receptor adaptations, such as desensitization, after chronic treatment (Smith *et al.*, 2007). Binding assays, such as [^{35}S]GTPγS provide important insight into the mechanisms through which combination treatments may be altering tolerance by directly evaluating agonist-stimulated MOR activation.

VII. Overall Scope of this Dissertation

The 5-HT$_{2C}$ receptor is expressed in regions that are known to modulate nociceptive responses and administration of 5-HT$_{2C}$ agonists are antinociceptive agents in preclinical models of *chronic pain* (Obata *et al.*, 2004; Nakai *et al.*, 2010; Ogino *et al.*, 2013). The development of more selective 5-HT$_{2C}$ receptor agonists, such as lorcaserin, provides the tools to further investigate how the activation of this receptor alters *acute pain-like responses* (which to date have not been investigated using this class of compound).

In the past several years, numerous studies have investigated the therapeutic potential of lorcaserin to alter the abuse-related effects of drugs of abuse in preclinical assays and yield conflicting results (Higgins *et al.*, 2012; Rezvani *et al.*, 2014; Banks and Negus, 2016; Harvey-Lewis *et al.*, 2016; Neelakantan *et al.*, 2017; Panlilio *et al.*, 2017). Despite the disparity in the data, the therapeutic use of lorcaserin in humans has progressed to several clinical trials investigating its effects on OUD in combination with naltrexone (ClinicalTrials.gov, 2017). In addition to understanding the means through which lorcaserin alters the abuse-related effects of opioids, it is important to understand how lorcaserin alters the antinociceptive effects of opioids as well.

The current status of the opioid epidemic supports the development of opioid-sparing analgesic combinations that serve to reduce the abuse-related effects and dose-dependent side effects associated with chronic opioid treatment. The analgesic properties of opioids are the most important component of their pharmacology and as further clinical development of lorcaserin as a treatment to prevent the development of opioid use disorder progresses, additional information investigating its effects on the antinociceptive effects of opioids is necessary. Based on the patterns from previous studies investigating opioids and lorcaserin, the hypotheses for the series of studies described herein is that lorcaserin will enhance the acute antinociceptive effects of opioids and attenuate the development of tolerance. To further develop these hypotheses, we employed three general aims:

1) Characterize the pharmacological effects of lorcaserin in a preclinical model of acute pain.
    a. Early preclinical studies administer 5-HT$_{2C}$ receptor agonists via intrathecal injection (see section on 5-HT$_{2C}$ agonists in the introduction for citations). Therefore, in a manner consistent with previously published data on 5-HT$_{2C}$ receptor agonists, lorcaserin was administered via the intrathecal route of administration for an appropriate basis of comparison.
    b. Further studies will compare its efficacy across other routes of administration and may provide insight into its general neuroanatomical locus of action.
2) Evaluate the effect of lorcaserin on the acute antinociceptive properties of opioids in the whole animal in a model of acute pain.
3) Evaluate the effect of chronic lorcaserin treatment on tolerance to the antinociceptive effects of oxycodone and determine the mechanisms through which the interaction may be occurring using previously validated *in vitro* models of tolerance.

Therefore, the overall goal of this dissertation is to characterize the effect of lorcaserin, a selective 5-HT$_{2C}$ receptor agonist, on both the acute and chronic properties of oxycodone. From these studies, we have characterized a novel, opioid-sparing target that should be investigated further for preclinical development that may provide alternative solutions to the current opioid epidemic.

Chapter 2

Characterization of the pharmacology of lorcaserin and its effects on acute opioid-induced antinociception

1. Summary

Opioids, such as morphine, oxycodone, fentanyl and methadone, are commonly used for the treatment of moderate to severe pain. Their use, even for short periods of time, present significant risks to the patient but these risks can be mitigated through use of multimodal adjunct therapies. Lorcaserin is a 5-HT$_{2C}$ receptor agonist that is shown to attenuate the abuse-related effects of oxycodone. The purpose of these studies was to characterize the effect of lorcaserin alone through several routes of administration and then evaluate its effects on acute opioid-induced antinociception. Intracerebroventricular lorcaserin was inactive but administration via intrathecal injection produced robust dose-dependent antinociception, suggesting a spinally-mediated mechanism of action. The spinal effects of lorcaserin were not blocked by naloxone pretreatment so the antinociceptive effects are not mediated through the endogenous opioid system. Subcutaneous injection of lorcaserin was inactive in the tail-withdrawal test. A combination treatment of subcutaneous lorcaserin and oral oxycodone produced a robust increase in both the potency and the time course of the opioid's activity. These effects were not blocked by naloxone but were antagonized by a 5-HT$_{2C}$ receptor antagonist. General behavioral depression is a concern in the evaluation of candidate analgesics, so the effect of lorcaserin on motor behavior was assessed. Lorcaserin did not alter the blood or brain concentrations of oxycodone, therefore its effects are not dependent upon changes in opioid metabolism. Agents, such as lorcaserin, may be useful adjunctive therapies for oxycodone in the treatment of acute pain.

II. Introduction

Opioids, such as oxycodone, fentanyl, and morphine, are commonly prescribed for the treatment of moderate to severe pain, but their chronic use presents serious risks to the patient, including the development of opioid use disorder (OUD) and overdose. Opioids produce their main pharmacological effects through the mu-opioid receptor (Sora *et al.*, 1997; Kitanaka *et al.*, 1998; Loh *et al.*, 1998). Increased prescription opioid misuse has led to the emergence of the opioid epidemic within the United States, and increased focus on developing alternative nonaddictive treatments for pain (CDC *et al.*, 2016, 2017; Volkow and Collins, 2017). Multimodal analgesia is a technique that seeks to improve pain-relief and reduce the incidence of side effects by optimizing the doses of analgesics in a manner that maximizes their efficacy (Buvanendran and Kroin, 2009; Buvanendran, 2011). Combination therapies aim to reduce the dose of opioid needed to achieve adequate pain relief while reducing overall risk to the patient.

Commonly used opioid-sparing adjuncts for the treatment of acute pain include nonsteroidal anti-inflammatory drugs (NSAIDs) and acetaminophen, which produce effects through inhibition of cyclooxygenase-1 (COX-1) and cyclooxygenase-2 (COX-2) enzymes (Huang *et al.*, 2008; Derry *et al.*, 2009, 2013; Gaskell *et al.*, 2009; Sullivan *et al.*, 2016). There are a number of opioid medications that have been formulated with NSAIDs or acetaminophen as a co-analgesic and the combinations are well-regarded in their ability to reduce the severity of pain (Derry *et al.*, 2009, 2013; Gaskell *et al.*, 2009). A major limitation of their utility is the risk of hepatoxicity or gastrointestinal bleeding associated with their prolonged use (James *et al.*, 2003; Nikolajsen *et al.*, 2006).

Tricyclic antidepressants (TCAs) and selective-serotonin reuptake inhibitors (SSRIs) are opioid-sparing treatments that are primarily used to treat chronic and neuropathic pain (Watson, 2000; Micó *et al.*, 2006; Dworkin *et al.*, 2007; Saarto and Wiffen, 2007; Dowell *et al.*, 2016a). Although opioids are no longer indicated as a first-line treatment for this population of patients, these drugs are effective in reducing the overall dose of opioid requirement (Watson, 2000; Dowell *et al.*, 2016a). Although antidepressants are mostly effective for treating chronic pain, their efficacy in treating acute pain is unclear (Gilron, 2016).

The serotonergic system is an important component in the elicitation of pain-relief and is proposed to exert its pharmacological effects through a descending modulatory pathway that directly modulates the activity of primary afferent neurons (Wilson *et al.*, 1979; Yahsh, 1979; Yezierski *et al.*, 1982; Takeuchi *et al.*, 1983; Jones and Light, 1990; Unit *et al.*, 1995; Millan, 1997; Cui *et al.*, 1999). Functional interactions between the opioid and serotonergic systems are noted and several studies demonstrate that the release of spinal serotonin partially underlies the antinociceptive effects of morphine (Ho *et al.*, 1975; Wilson *et al.*, 1979; Yaksh and Tyce, 1979; Crisp *et al.*, 1991; Schul and Frenk, 1991; Jolas *et al.*, 1999).

The serotonergic system is composed of over 14 different subtypes and the serotonin 2c receptor (5-HT_{2C}) has emerged as a novel target for treating drug addiction, neuropsychiatric diseases, and pain (Hoyer *et al.*, 1994; Bubar and Cunningham, 2008; Vincenzo, 2015). 5-HT_{2C} receptor agonists have demonstrated preclinical efficacy in rodent models of fibromyalgia and neuropathic pain (Obata *et al.*, 2004; Nakai *et al.*, 2010; Ogino *et al.*, 2013). Lorcaserin is a selective 5-HT_{2C} receptor agonist that possesses 15-fold greater selectivity for the 5-HT_{2C} receptor than 5-HT_{2A} receptor (Thomsen *et al.*, 2008). Lorcaserin was originally developed as a pharmacotherapeutic treatment for obesity but in recent years evaluated as a possible treatment for drug addiction (Smith *et al.*, 2009; Fidler *et al.*, 2011; GT Collins *et al.*, 2017).

The current literature suggests that lorcaserin may function as a favorable opioid-sparing adjunct as it reduces the abuse-related effects of opioids and may produce antinociception through alternative, non-opioid-dependent mechanisms (Nakai *et al.*, 2010; Ogino *et al.*, 2013; Wu *et al.*, 2015; Zhang *et al.*, 2015; Neelakantan *et al.*, 2017). In the study by Nakai *et al.* (2010), lorcaserin attenuated mechanical hypersensitivity in a rodent model of fibromyalgia. Previous research has exclusively evaluated the effect of 5-HT$_{2C}$ agonists in models of chronic pain. The aim of these studies was to evaluate the effect of lorcaserin in a model of acute pain and its potential as an opioid-sparing analgesic in this model.

III. Materials & Methods

Drugs and Chemicals. Oxycodone hydrochloride and methadone hydrochloride (National Institutes on Drug Abuse, Bethesda, MD) were prepared in pyrogen-free isotonic saline (Hospira, Lake Forest, IL) and administered via oral gavage (p.o.). Morphine sulfate and fentanyl (National Institute on Drug Abuse, Bethesda, MD) were dissolved in pyrogen-free isotonic saline and administered subcutaneously (s.c.). Lorcaserin hydrochloride and SB242084 were purchased from Cayman Chemicals (Ann Arbor, MI). Lorcaserin was prepared in isotonic saline to be injected s.c. SB242084 was prepared in a mixture of 8% by volume 2-hydroxypropyl-β-cyclodextrin in saline. WAY163909 was generously provided by Dr. Kathryn Cunningham and Mr. Robert Fox of the University of Texas Medical Branch (Galveston, TX) and prepared in saline. Drugs prepared for intracerebroventricular and intrathecal injections were prepared in deionized water (in house). Drugs for i.c.v. and i.t. were not prepared in saline due to the adverse effects of saline when administered via i.t. and i.c.v..

Subjects. Male, Swiss Webster mice (8 – 10-week-old, Harlan Laboratories, Indianapolis, IN) weighing 25 – 35g were housed in community cages in the animal care facilities (22 ± 2°C, 12-

hour light-dark cycle) with a*d libitum* access to food and water. On the day prior to experimentation, the mice were moved to the laboratory and allowed to acclimate overnight. Animal care and experimental procedures were performed according to an Institutional Animal Care and Use Committee (IACUC) approved protocol at Virginia Commonwealth University.

Intracerebroventricular Injections. Intraventricular injections were performed as described by Pedigo *et al.* (1975). Mice were anaesthetized with 2.5% isoflurane before a transverse incision was made in the scalp. Mice were allowed to recover for at least two hours after surgery. A free hand 5µL injection of the drug or vehicle was made 2mm rostral and 2mm lateral at a 45° angle from the bregma into the lateral ventricle. The extensive experience of this laboratory has made it possible to inject drugs with greater than 95% accuracy. Immediately after testing, animals were euthanized to minimize excessive distress, according to IACUC protocols. Antinociceptive testing was conducted 10 minutes after intracerebroventricular administration.

Intrathecal Injections. Intrathecal injections were performed according to the protocol of Hylden and Wilcox (1983). Unanesthetized mice were injected with a volume of 5 µL between the L5 and L6 area of the spinal cord using a 30-gauge, ½-inch needle. Based on the time course experiments of lorcaserin's intrathecal activity, all antinociceptive testing was conducted 10 minutes after intrathecal injection.

Warm Water Tail-Withdrawal Test. The warm water tail withdrawal test used to assess antinociception in mice was developed by D'Amour and Smith (1941) but modified by Dewey et al (1970). In all experiments (unless otherwise stated), mice were tested using a 52° C water bath. Before drug administration, the baseline (control) latency for each mouse was determined and only mice with a control reaction time from 2 – 4 seconds were used. The test latency after drug treatment was assessed 20 minutes after drug administration, with a maximum cut-off value of 10 seconds to prevent tissue damage to the tail. Antinociception was quantified according to the method of Harris & Pierson (1964) as the percentage of maximum possible effect (%MPE) which was calculated ad: %MPE = [(test control – control)/(10 – control)]x100.

Experimental Design for cumulative dosing protocol. Drugs were administered using a cumulative dosing technique. In the drug-combination studies, saline or lorcaserin were administered at doses of 0.25, 0.5, 1, 2, and 4 mg/kg (s.c.), 30 minutes prior to the first opioid treatment. After lorcaserin pretreatment, the first dose of opioid was administered via oral gavage or subcutaneous injection and animals were tested 20 minutes later. After each round of testing, animals received an additional cumulative dose of opioid and tested again 20 minutes later. Testing and dosing continued until the animal reached the maximum cut-off time of 10 seconds.

Time Course Experiment. The warm-water tail withdrawal test used to evaluate the effect of lorcaserin on the time-course of oxycodone. Mice were first administered saline or lorcaserin (0.5 or 1 mg/kg, s.c.), 30 minutes prior to opioid treatment. After the lorcaserin pretreatment, mice were administered saline or oxycodone (10 mg/kg, p.o.) and then tested at the following time points: 15, 30, 60, 120 minutes for the tail flick latency response times. For studies utilizing the 5-HT$_{2C}$ receptor antagonist, SB242084, mice were injected 10 minutes before lorcaserin treatment. All other drug treatments and time points remained the same.

Locomotor Activity Studies. The motor effects of lorcaserin were assessed using measurements of locomotor activity. Locomotor activity was assessed in enclosed, sound attenuating, photo beam activity monitors (Med Associates., St. Albans, VT) that record “ambulatory counts” via photo beam breaks. Numbers of beam breaks were recorded in 5-minute time blocks. Mice were administered saline or lorcaserin (0.5, 1, or 2 mg/kg, s.c.) and immediately placed in the chamber for 40 minutes of recording. Activity chambers were thoroughly cleaned between subjects with cleaning solution and then dried. In studies using lorcaserin and oxycodone, mice were administered lorcaserin 30 minutes before treatment with oxycodone (64 mg/kg, p.o.) and then transferred to the activity cages 20 minutes after oxycodone treatment.

Naloxone Antagonism and Cumulative Oxycodone Dosing Study. The warm-water tail withdrawal test was used to evaluate the effect of lorcaserin on naloxone-antagonism of oxycodone-induced antinociception. Lorcaserin (1 mg/kg, s.c.) was administered 30 minutes before the first cumulative dose of oxycodone. 5 minutes before the first dose of oxycodone was administered, naloxone (1 mg/kg, s.c.) was injected. Mice were tested 20 minutes after the administration of oxycodone for antinociceptive responses. After each round of testing, animals received an additional cumulative dose of oxycodone and were tested 20 minutes later. This process was repeated until animals reached the cut-off time of 10 seconds.

5-HT$_{2A}$ Knockout Animals. Experiments were performed on adult (10- to 14-week-old) male mice. 5-HT$_{2A}$ receptor knockout mice of 129S6/Sv background have been previously described (González-Maeso *et al.*, 2003). For experiments using genetically modified mice, wild-type controls purchased from Taconic Biosciences (Rensselaer, NY). Morphine dose-response curves were generated using a cumulative dosing protocol as previously described above and nociceptive testing was conducted using the warm-water tail withdrawal test at 56°C.

Oxycodone Distribution Experiments. Tissues were dissected from mice that were treated with oxycodone (10 mg/kg, p.o.) and/or lorcaserin (2 m/kg, s.c.). Mice were administered oxycodone (10 mg/kg, p.o.) and/or lorcaserin (2 mg/kg, s.c.) and then dissected 30-minutes or 120-minutes after drug administration. After dissection, tissues were homogenized in 1:3 ratio of brain tissue (mg): deionized water (mL). The quantification of oxycodone was performed using Ultra performance liquid chromatography tandem mass spectrometer (UPLC-MS/MS) method. An oxycodone seven-point calibration curve at concentrations of 10 -1000 ng/mL for blood and 10 – 1000 ng/kg for brain tissue homogenate and negative controls with or without internal standard (ISTD) were prepared in drug-free mouse blood and brain tissue with each analytical run. Oxycodone was extracted from blood and brain tissue homogenate using an ISOLUTE® PLD+ Protein and Phospholipid Removal 96 well plate. In brief, the ISTD, 10 ng of oxycode-d6, was added to aliquots of 100 µL of blood or 400 µL of homogenized brain tissue calibrators, controls and samples. These samples were mixed and allowed to equilibrate. 0.4 mL acetonitrile was added to the extraction chambers in the plate. The samples were then dispensed with force and allowed to mix for 5 mins. Samples were then eluted at 2-4 psi under nitrogen in to a 96 well plate for analysis using a UCT Positive Pressure Manifold (Bristol, PA) for analysis.

The Ultra performance liquid chromatography tandem mass spectrometer (UPLC-MS/MS) analysis was performed on Waters AcQuity XEVO-TQ-S Micro UPLC-MS/MS system (Milford, Massachusetts). Chromatographic separation of Oxycodone and the ISTD, oxycodone d6, was performed using Restek Ultra Biphenyl 3um, 100 x 2.1 mm column (Bellefonte, PA). The mobile phase contained A (20 mM ammonium formate in water) and B (20 mM ammonium formate in methanol) and was delivered at a flow rate of 0.6 mL/min with the following gradient: 95% A changed to 60 at 1.5 mins. Then ramped to 100% B and held for 0.5 mins and returning to 95% B at 3.6 mins. The source temperature was set at 150°C with a desolvation temperature of 500°C. The cone flow rate was 100 L/hr and the desolvation gas had a flow rate of 40°C L/H. The acquisition mode used was multiple reaction monitoring (MRM). The following transition ions were monitored in positive mode: 316>241 & 316>212 for oxycodone and 322>247 & 322>218 for oxycodone-d6. The total run time for the analytical method was 4.0 minutes.

Lorcaserin Distribution Experiments. The quantification of lorcaserin was performed using an Ultra performance liquid chromatography tandem mass spectrometer (UPLC-MS/MS). A lorcaserin seven-point calibration curve at concentrations of 10 -1000 ng/mL for blood and 10 – 1000 ng/kg for brain tissue homogenate and negative controls with or without internal standard (ISTD) were prepared in drug-free mouse blood and brain tissue with each analytical run. Lorcaserin was extracted from blood and brain tissue homogenate using the addition of acetonitrile. In brief, the ISTD, 10ng of cocanine-d3, was added to aliquots of 100 µL of blood or 400 µL of homogenized brain tissue calibrators, controls and samples. These samples were mixed and allowed to equilibrate. 0.2 mL acetonitrile was added to each sample and vortex mixed. The samples were then centrifuged at 3500 rpm for 10 min. After centrifuging the top layer containing the acetonitrile was removed and placed in auto-sampler vials for analysis.

The Ultra performance liquid chromatography tandem mass spectrometer (UPLC-MS/MS) analysis was performed on a Sciex 6500 QTRAP system with an IonDrive Turbo V source for TurbolonSpray® (Sciex, Ontario, Canada) attached to a Shimadzu UPLC system (Kyoto, Japan) controlled by Analyst software (Sciex, Ontario, Canada). Chromatographic separation of lorcaserin and the ISTD, cocaine d3, was performed using a Thermo Hypersil Gold column, 50 x 2.1 mm, 3 micron (Thermofisher Scientific, USA). The mobile phase contained water/methanol (40:60, v/v) with 0.1 mM ammonium formate and was delivered at a flow rate of 1 mL/min. The source temperature was set at 600°C, and curtain gas had a flow rate of 30 mL/min. The ionspray voltage was 5500 V, with the ion source gases 1 and 2 having flow rates of 60 and 45 mL/min, respectively. The acquisition mode used was multiple reaction monitoring (MRM). The following transition ions were monitored in negative mode: 196>144 & 196>129 for lorcaserin and 307>185 & 307>105 for cocaine-d3. The total run time for the analytical method was 2.0 minutes.

Data Analysis. Opioid dose-response curves were constructed for determination of ED_{50} values by the Bliss (1967) method, using least-squares linear regression analysis followed by calculation of 95% confidence limits. All other statistical analysis were conducted in GraphPad Prism 5 software (GraphPad Software, La Jolla, CA) and all data are presented as the mean ± standard error of the mean. In comparisons between three or more groups with a single factor, a one-way analysis of variances (ANOVA) with Tukey's post-hoc analysis was used. In two or more groups of data, statistical differences were analyzed using the Student's two-tailed unpaired *t*-test. Differences were considered significant when $P < 0.05$ and when ED_{50} confidence limits did not overlap.

IV. Results

Subcutaneous Lorcaserin and Opioid antinociception

Dose response effect of lorcaserin on opioid antinociception. Lorcaserin alone was inactive in the warm-water tail withdrawal test, up to doses of 8 mg/kg (Figure 2.1). A range of doses (0.25 – 4 mg/kg, s.c.) of lorcaserin were tested for their effect following administration of cumulative doses of oral oxycodone (Figure 2.2). At all doses tested, there was an observable shift to the left of the oxycodone dose-response curve. Acute oxycodone alone produced an ED_{50} of 8.39 mg/kg (7.30 – 9.65) and significant shifts of the curve were produced by 2 and 4 mg/kg lorcaserin (see Table 2.1). Shifts were considered significant when 95% confidence limits did not overlap.

Morphine produced an ED_{50} of 4.19 mg/kg (3.23 – 5.43) in control mice. Pretreatment with 1 or 2 mg/kg lorcaserin shifted the curves to the left (Figure 2.3A). An acute injection of fentanyl (Figure 2.3B) produced an ED_{50} value of 57.64 ug/kg (47.67 – 69.69) and pretreatment with a dose of 1 mg/kg lorcaserin produced a significant shift of the ED_{50} to 33.52 μg/kg (26.75 – 42.02). The effect of subcutaneous lorcaserin on methadone was also characterized (Figure 2.4) but at all doses tested, lorcaserin did not shift the dose-response effect.

WAY163909 on oxycodone-induced antinociception. In order to determine if the effect of lorcaserin was due to some other effect other than activation of the 5-HT$_{2C}$ receptor, an additional 5-HT$_{2C}$ receptor agonist, WAY163909, was tested for its effect on oxycodone-induced antinociception (Figure 2.5). Control mice that received saline produced an ED$_{50}$ value of 6.83 mg/kg (5.30 – 8.81). Treatment with WAY163909 produced a significant shift at 1 mg/kg with an ED$_{50}$ of 3.35 mg/kg (2.20 – 5.10) and treatment with 2 mg/kg WAY163909 produced an ED$_{50}$ of 4.05 mg/kg (2.42 – 6.79).

Antagonism Studies. The potentiating effect of lorcaserin on oxycodone antinociception was not antagonized by naloxone (Figure 2.6). Mice were administered pretreatments of saline only, lorcaserin (1 mg/kg, s.c.) and saline, saline and naloxone (1 mg/kg, s.c.), or lorcaserin and naloxone. Control animals that received only saline prior to oxycodone dosing produced an ED$_{50}$ of 9.07 mg/kg (7.23 – 11.38) and pretreatment with naloxone produced a significant 2-fold shift in the ED$_{50}$ to 17.81 mg/kg (13.23 – 23.99). Treatment with lorcaserin alone produced a shift in the ED$_{50}$ to 7.34 mg/kg (5.62 – 9.60). Lorcaserin blocked antagonism by naloxone and produced an ED$_{50}$ of 8.23 mg/kg (6.40 – 10.58) which is similar to control (oxycodone alone) mice. The inability of naloxone to block the enhancement of oxycodone antinociception by lorcaserin is consistent with an earlier study where naloxone was similarly unable to block the antinociceptive effect of intrathecal lorcaserin.

The selective 5-HT$_{2C}$ receptor antagonist, SB242084, was tested against the antinociceptive effect of oxycodone (Figure 2.7) and was inactive at all doses test (0.5,1 and 2 mg/kg, i.p.). Two-way ANOVA with Dunnet’s multiple comparison test revealed that there was no main effect of pretreatment with SB242084 [F (3, 155) = 1.969, *P* = 0.1209].Mice treated with oxycodone alone produced an ED_{50} value of 8.50 mg/kg (7.16 – 10.08). Pretreatment with 0.5 mg/kg SB242084 produced an ED_{50} value of 6.99 mg/kg (5.49 – 8.77). Pretreatment with 1 mg/kg SB242084 produced an ED_{50} value of 8.79 mg/kg (7.16 – 10.08) and preareatment with 2 mg/kg SB242084 produced an ED_{50} value of 6.43 mg/kg (5.34 – 7.74).

The effect of genetic deletion of the 5-HT_{2A} receptor on morphine-induced antinociception and lorcaserin treatment. Mice with a global knockout of the 5-HT_{2A} receptor were tested to assess the contributions of the 5-HT_{2A} receptor on the antinociceptive effects of morphine and the combined treatment of lorcaserin and morphine (Figure 2.8). Wild-type mice treated with cumulative morphine produced an ED_{50} of 1.73 mg/kg (1.38 – 2.18) and in 5-HT_{2A} receptor KOs produced an ED_{50} of 0.95 mg/kg (0.56 – 1.61), trending towards a significant ED_{50} shift. Pretreatment with lorcaserin (2 mg/kg, s.c.) in the WT mice produced an ED_{50} of 1.15 mg/kg (0.90 – 1.46 mg/kg) and in the KO mice, an ED_{50} of 0.66 mg/kg (0.39 – 1.11).

The effect of subcutaneous lorcaserin on the time course of oxycodone. Lorcaserin's effect on oxycodone's time course of activity was evaluated (Figure 2.9). Mice were pretreated with a dose of lorcaserin (0.5 or 2 mg/kg, s.c.) and then 30 minutes later, administered an approximate ED_{50} dose of oxycodone (10 mg/kg, p.o.). The control animals that only received oxycodone displayed peak antinociceptive activity at 30 minutes and returned to baseline tail withdrawal latency values by 60 minutes. 2 mg/kg lorcaserin pretreatment [F (1, 16) = 17.77, P=0.0007] produced significant shifts in oxycodone efficacy relative to control values at time points 15, 30 and this effect persisted up to 60 minutes [$P < 0.005$] (15 and 60 minutes) and $P < 0.05$ (15 minutes), two-way ANOVA with Dunnett's multiple comparisons test]. Mice that received a subthreshold dose of lorcaserin (0.5 mg/kg, s.c. [F (1, 26) = 3.542, *P* = 0.0711]) displayed a significant potentiation at the 60-

minute time point ($P < 0.05$, two-way ANOVA with Dunnett's multiple comparisons test). These data indicate that lorcaserin alters both the acute potency of opiates, as well as its time course of activity.

Furthermore, the data demonstrate that the enhancing effect of lorcaserin (2 mg/kg, s.c.) was blocked by administration of SB242084, a 5-HT_{2C} receptor antagonist (Figure 2.10). Relative to control mice that only received oxycodone (10 mg/kg, p.o.), pretreatment with lorcaserin (2 mg/kg, s.c., [F (1,17) = 29.77, $P<0.0001$]) significantly extended the time course of oxycodone's activity at all time points tested ($P < 0.001$, two-way ANOVA with multiple comparisons and Sidak posthoc test). SB242084 (1 mg/kg, i.p.; [F(1,17) = 18.06, $P < 0.0005$]) significantly blocked the effect of lorcaserin relative to mice that received lorcaserin and oxycodone at the 60- and 120-minute time points ($P < 0.05$, two-way ANOVA with multiple comparisons) and were not significantly different from mice that received oxycodone alone [F(1, 18) = 1.365, $P = 0.257$] . SB242084 alone did not significantly alter the time course of oxycodone's antinociceptive activity [F (1, 17) = 0.6833, $P = 0.4199$].

Effect of subcutaneous lorcaserin on motor activity. To assess the potential of lorcaserin to produce general motor depressant effects which may confound observed antinociceptive effects, lorcaserin was tested for its effects on locomotor activity (Figure 2.11). The data in Figure 3 are presented as total ambulatory counts per 40-minute testing period. Saline treated mice displayed a mean ambulatory count value of 3801 (S.E.M. = 570.4). Treatment with lorcaserin (1 and 2 mg/kg, s.c.) significantly reduced total ambulatory counts to 1508 (S.E.M. = 321.0) and 1603 (S.E.M. = 184.4) counts ($P < 0.001$, one-way ANOVA) respectively. The low dose of lorcaserin (0.5 mg/kg, s.c.) did not significantly attenuate motor activity and presented a mean count of 2425 (S.E.M. = 244.9).

The effect of intrathecal lorcaserin on oxycodone antinociception. Interactions between i.t. lorcaserin and oral oxycodone in the warm-water tail withdrawal test were evaluated (Figure 2.12). First, the antinociceptive effect of lorcaserin alone was characterized. Intrathecal lorcaserin (Figure 2.12A) produced robust dose-dependent antinociception and significant antinociceptive effects were observed at 64 and 128 µg ($P < 0.001$, one-way ANOVA) compared to control mice. The ED_{50} value for intrathecal lorcaserin was 54 µg. Intrathecal lorcaserin displayed a quick onset of activity, with peak effect occurring at 5 – 10 minutes, and then rapidly returned to normal baseline values (Figure 2.12B). The antinociceptive effect of intrathecal lorcaserin (Figure 2.12C) was not blocked by pretreatment with the opioid antagonist, naloxone (1 mg/kg, s.c.).

Following this characterization, in Figure 2.12D, mice were administered a dose of oxycodone (10 mg/kg, p.o.) and then given an intrathecal injection of a subthreshold dose of lorcaserin (32 µg). The intrathecal dose of lorcaserin (32 µg) did not produce a behaviorally significant effect on its own and modestly increased the efficacy of the oxycodone treatment but not in a significant manner ($P = 0.0692$, one-way ANOVA).

Intracerebroventricular Lorcaserin and Oxycodone antinociception. The effect of intracerebroventricular (i.c.v.) lorcaserin on oxycodone antinociception was similarly evaluated (Figure 2.13). Intracerebroventricular lorcaserin alone was behaviorally inactive at all doses tested and did not display any antinociceptive activity at any point during the time course study (Figure 2.13A and 2.13B). Some mice that were injected with lorcaserin (128 µg, i.c.v.) exhibited spontaneous seizure activity and were not used for antinociceptive testing. The data presented in Figure 2.13C demonstrated the effect of lorcaserin (i.c.v., 64 µg) on an ED_{80} dose of oxycodone (16 mg/kg, p.o.), where lorcaserin significantly attenuated the acute antinociceptive effect of oral oxycodone ($P < 0.05$, Student's two-tailed unpaired *t*-test). These data and the intrathecal lorcaserin data demonstrate differences in the spinal and supraspinal routes of administration and their effects on opioid antinociception.

Biodisposition of SC lorcaserin in the mouse and its effects on oxycodone distribution. The concentrations of lorcaserin in the whole brain, spinal cord, and blood after subcutaneous administration are presented in Figure 2.14. Administration of lorcaserin resulted in a notably elevated accumulation in the brain and spinal cord tissue relative to the blood by an approximately ~20-fold difference. Lorcaserin (2 mg/kg, s.c.) did not alter the blood or brain concentrations of oxycodone at either 30 and 120-minute intervals (Figure 2.15).

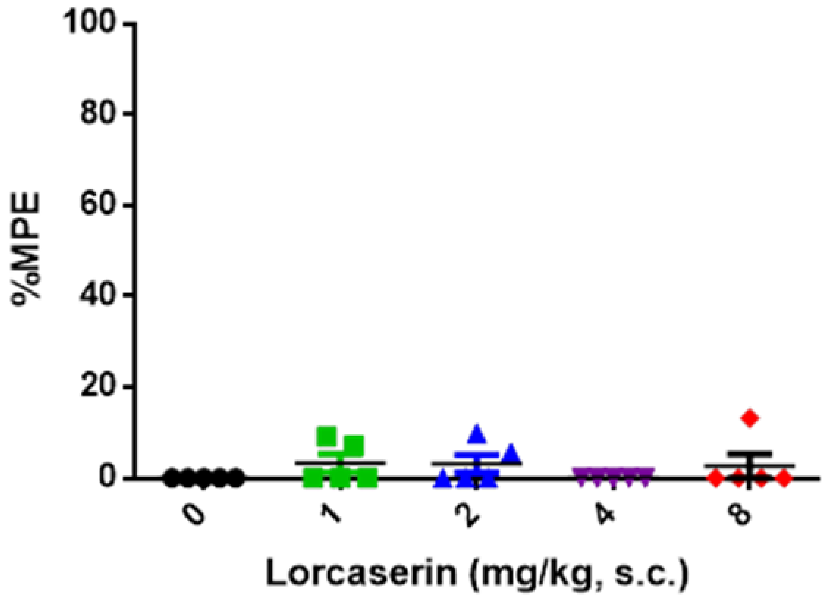

Figure 2.1: Subcutaneous lorcaserin is inactive in the warm-water tail-withdrawal assay. Administration of lorcaserin (up to 8 mg/kg, s.c.) did not produce antinociceptive effects in the tail withdrawal assay. Each point was generated with five mice and data points are represented as the mean ± S.E.M.

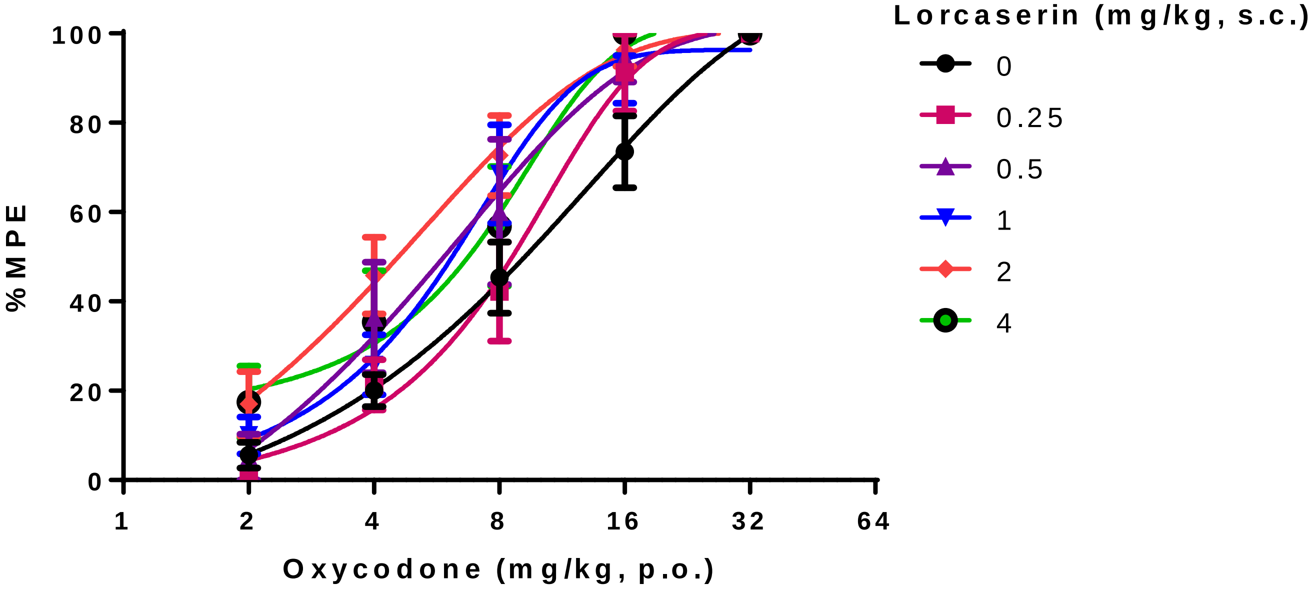

Figure 2.2 Lorcaserin pretreatment shifts the dose-response curves of cumulatively administered oxycodone. At all doses tested, lorcaserin treatment produced shifts of the dose-response curves to the left, with significant shifts in the ED_{50} values (relative to saline controls) occurring with 2 and 4 mg/kg lorcaserin. Data points are represented by the mean ± S.E.M.

Opioid	Lorcaserin (mg/kg)	ED_{50} (mg/kg)	+/- CL	Potency Ratio
Oxycodone	0	8.39	7.30 – 9.65	---
Oxycodone	0.25	7.75	6.50 – 9.23	1.06 (0.82 – 1.38)
Oxycodone	0.5	6.17	4.80 – 7.95	1.34 (0.99 – 1.82)
Oxycodone	1	6.22	5.23 – 7.40	1.32 (1.06 – 1.65)
Oxycodone	2	4.73*	3.88 – 5.77	1.69 (1.69 – 2.15)
Oxycodone	4	5.58*	4.33 – 7.18	1.45 (1.10 – 1.94)

*Significant shift in the ED_{50}, determined by confidence limits that do not overlap

Table 2.1: Comparison of the ED_{50} values of oral oxycodone with and without subcutaneous lorcaserin pretreatment. ED_{50} values and the 95% confidence limits were generated using a cumulative dosing protocol in mice. Oral oxycodone produced dose-dependent antinociception following repeated administration. Lorcaserin produced significant shifts in the observable ED_{50} of oxycodone.

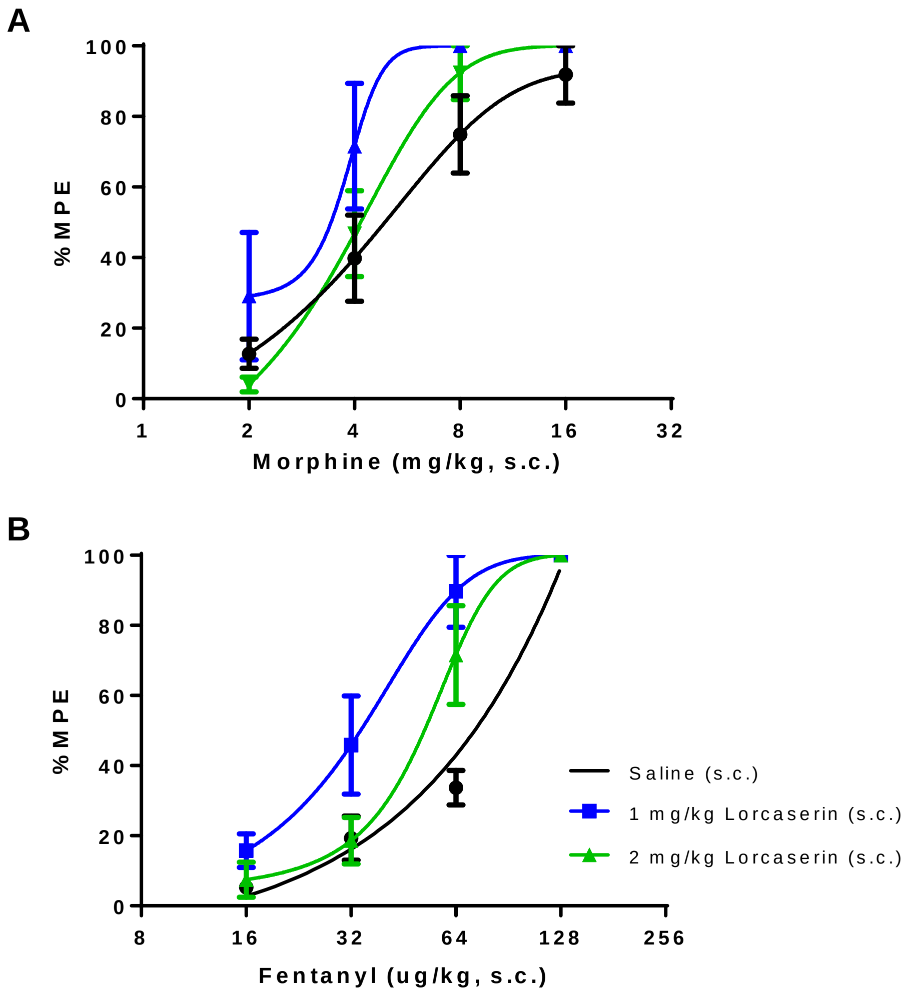

Figure 2.3: The administration of subcutaneous lorcaserin potentiated the acute antinociceptive effect of fentanyl and morphine. Animals were tested using the cumulative dosing protocol and both fentanyl and morphine were administered subcutaneously. Prior to opioid administration, animals received an injection of lorcaserin and then thirty minutes later, the first dose of opioid was administered. Behavioral testing occurred twenty minutes after each opioid dose and from this data, dose-response curves were constructed and ED_{50} values were generated. Lorcaserin (1 mg/kg, s.c.) produced significant shifts in the ED_{50} values of morphine and fentanyl. Each data point is represented by a minimum of five animals and each individual point is represented as the mean ± S.E.M.

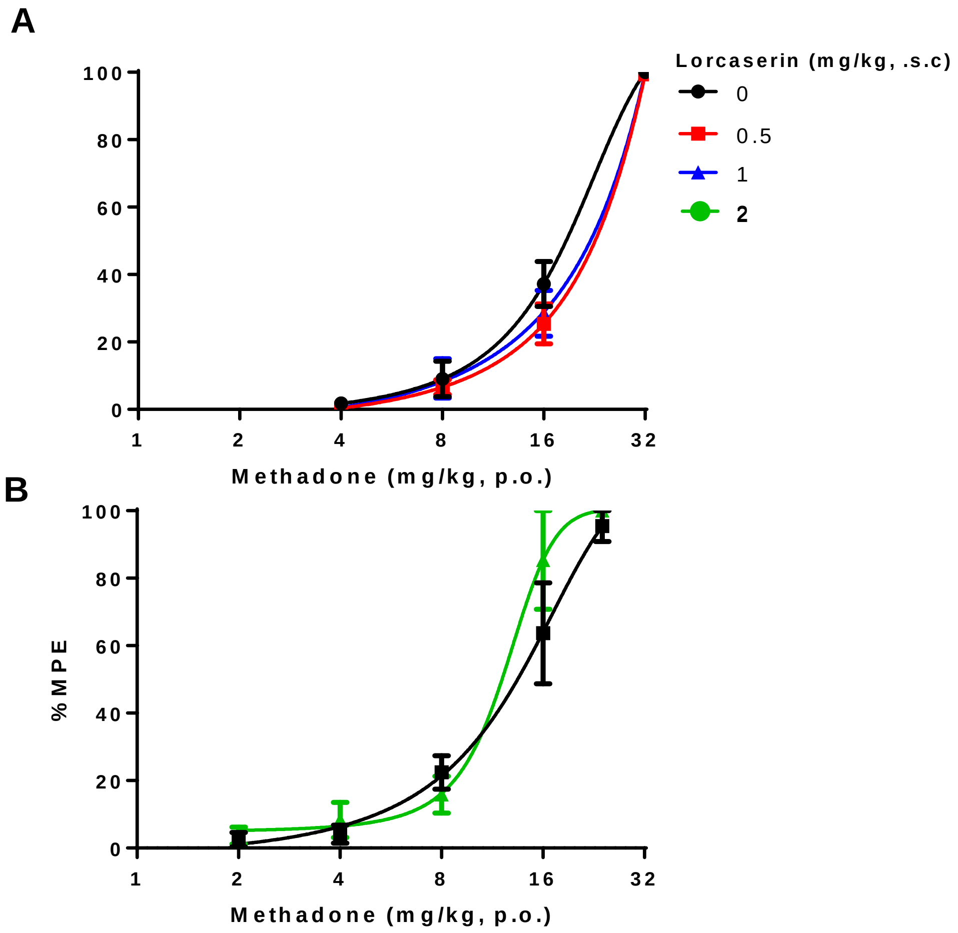

Figure 2.4: Lorcaserin did not enhance the antinociceptive effects of orally administered methadone. Mice were treated with lorcaserin (0.5, 1, or 2 mg/kg, s.c.) and then administered cumulative doses of oral methadone[1]. The resultant ED_{50} values of all groups did not significantly differ from their controls. A minimum of 5 mice were used per curve and data are expressed at the mean ± S.E.M.

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[1] The curves generated for methadone are on separate graphs because the final tested doses are different (i.e., 24 mg/kg vs. 32 mg/kg). After noticing that the ascending portion of the curve was extremely steep after 16 → 32 mg/kg, we chose a dose (24 mg/kg) on the intermediate portion of that ascending curve for additional testing.

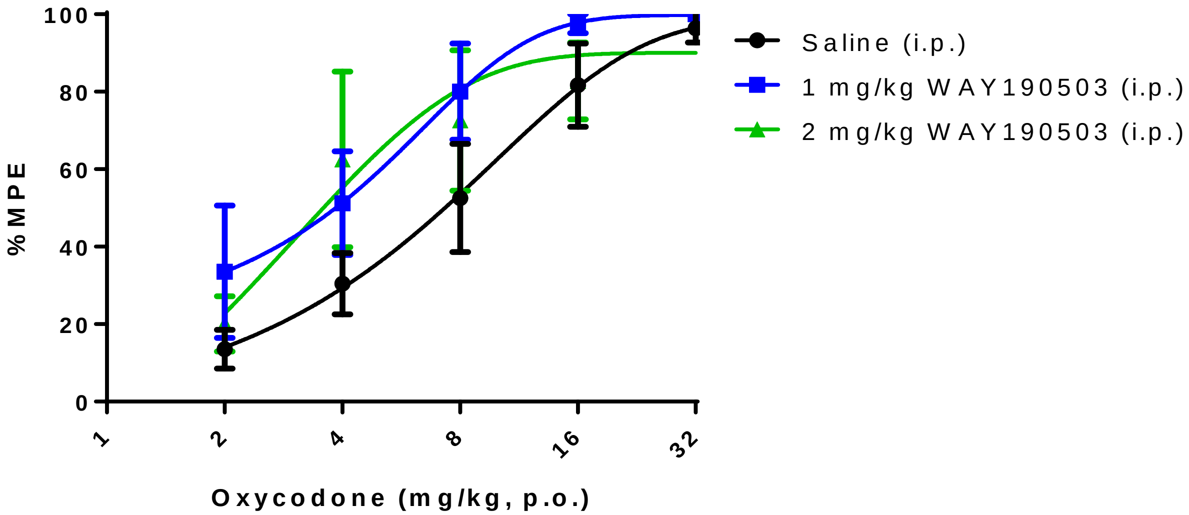


Figure 2.5: Administration of WAY 163909, another selective 5-HT$_{2C}$ receptor agonist, enhanced the antinociceptive effect of oxycodone. Mice were administered intraperitoneal injections of WAY163909 (1 or 2 mg/kg, i.p.) and then administered cumulative doses of oxycodone. WAY163909 produced significant shifts in the oxycodone dose response curve. Each data point is represented by a minimum of five mice and displayed as the mean ± S.E.M.

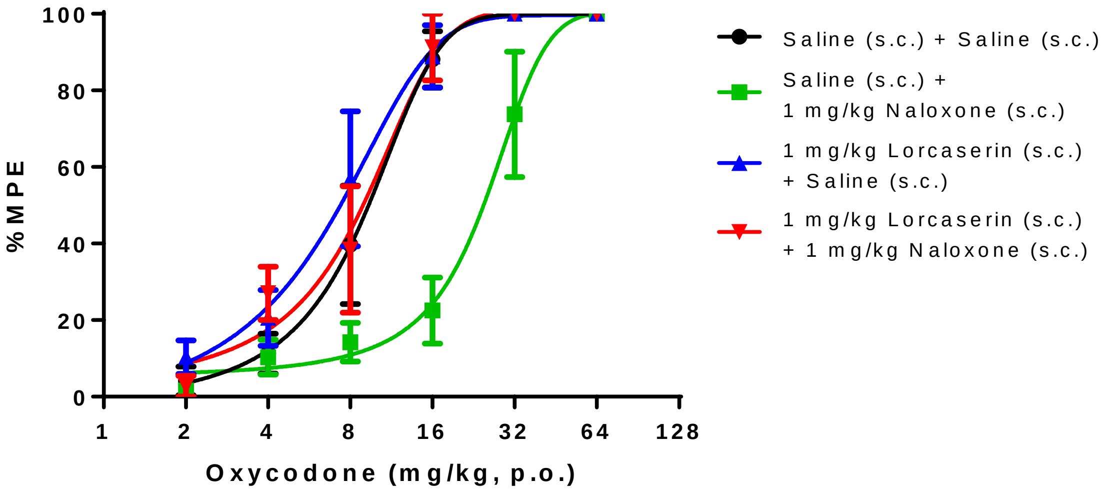


Figure 2.6: The effect of lorcaserin on oxycodone antinociception was not antagonized by naloxone. Animals were pretreated with a s.c. injection of lorcaserin (1 mg/kg). Approximately 5 minutes before the first oxycodone treatment, animals were administered a dose of (1 mg/kg, s.c.). A minimum of 5 mice were used per curve and each data point is represented as the mean ± SEM.

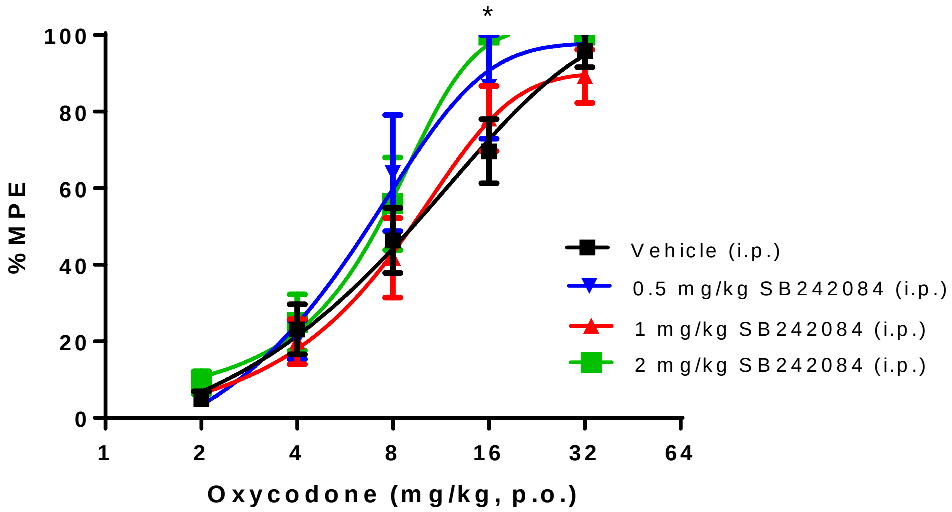


Figure 2.7: The effect of SB242084, a selective 5-HT$_{2C}$ receptor antagonist, on the acute antinociceptive effect of oxycodone. Pretreatment with SB242084 (0.5, 1, or 2 mg/kg, i.p.) did not significantly alter the ED$_{50}$ value of acute oxycodone. Treatment with 2 mg/kg SB242084 significantly enhanced the acute antinociceptive effect of 16 mg/kg oxycodone ($P < 0.05$, Two-way ANOVA with multiple comparisons). A minimum of 5-10 mice were used per curve and each data point is represented as the mean ± S.E.M.

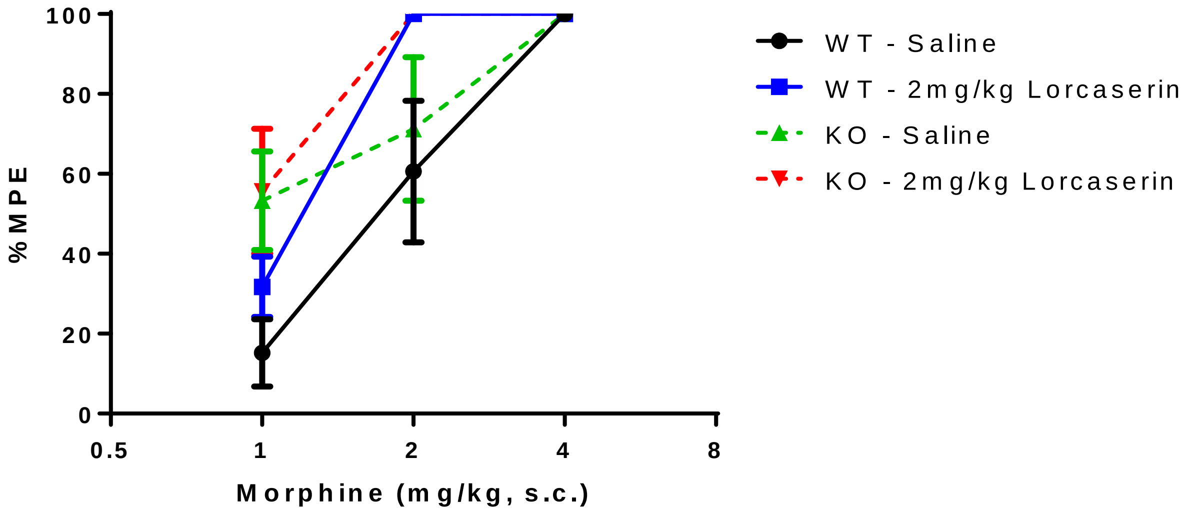


Figure 2.8: Genetic knockout of the 5-HT$_{2A}$ receptor did not alter the enhancing effect of lorcaserin on opioid antinociception. Mice were pretreated with lorcaserin (2 mg/kg, s.c.) and then administered cumulative doses of morphine. Each point is represented by the mean of 5 animals ± S.E.M.

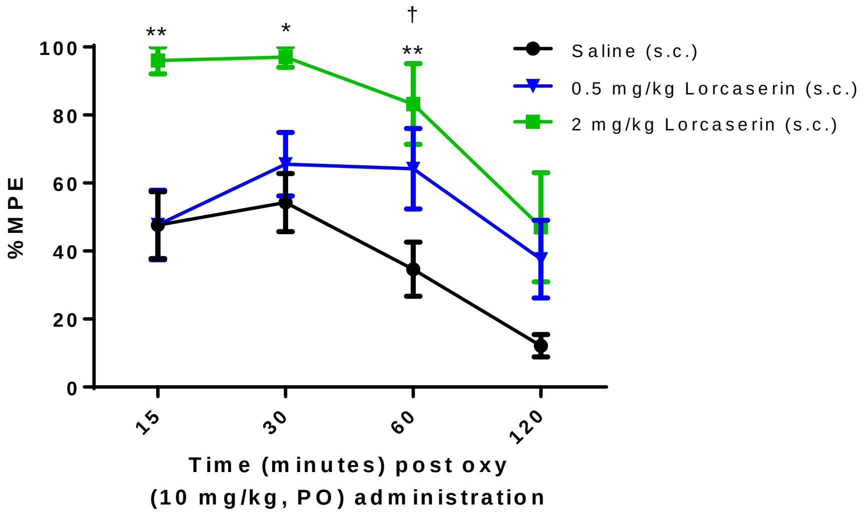


Figure 2.9: The subcutaneous administration of lorcaserin altered the time course of oxycodone. 2 mg/kg lorcaserin produced the greatest enhancement with significant effects observed up to 60 minutes (*P<0.05, ** P < 0.005, two-way ANOVA). The subthreshold dose of lorcaserin similarly produced an enhancement of oxycodone but was only significant at 60 minutes (†P<0.05, two-way ANOVA). A minimum of 5-10 mice per group with the data shown as the mean ± S.E.M.

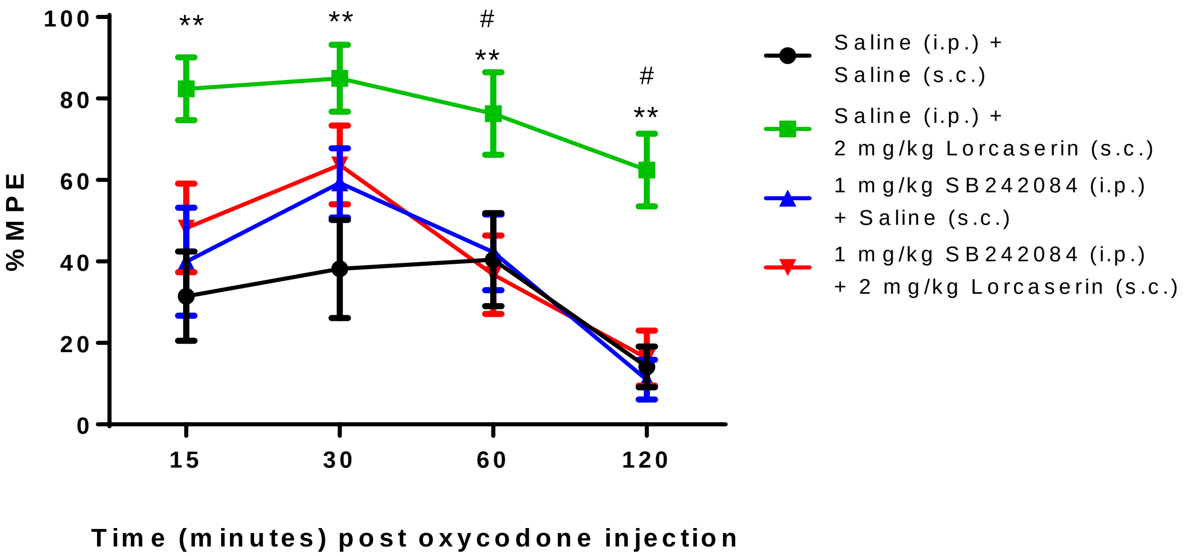
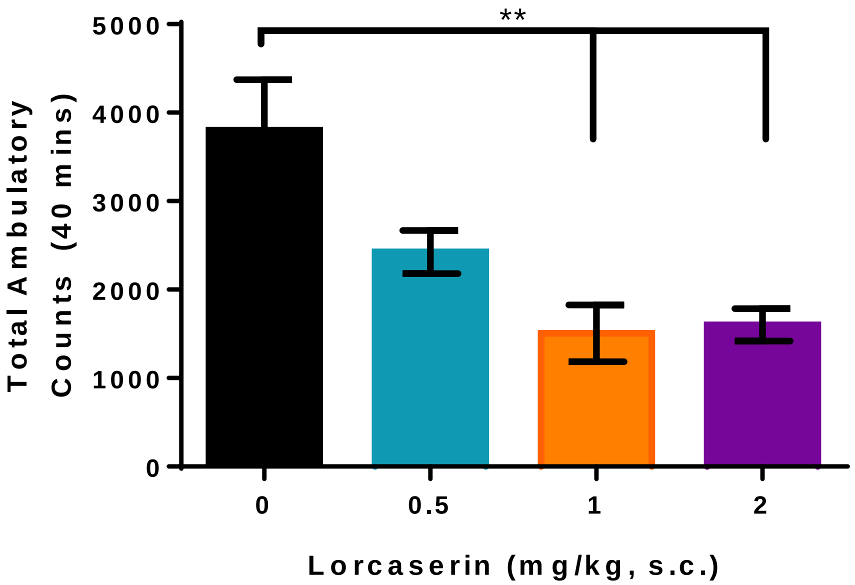


Figure 2.10: Treatment with SB242084, a selective 5-HT$_{2C}$ receptor antagonist, blocked the enhanced antinociceptive time course of lorcaserin and oxycodone. Lorcaserin significantly enhanced the time course of oxycodone relative to saline controls at the at all time points (***P* < 0.005, 2-way ANOVA with multiple comparisons). Treatment with SB242084 did not significantly alter the time course of oxycodone relative to control mice that received oxycodone alone. SB242084 significantly antagonized the enhanced effect of opioid antinociception by lorcaserin at the 60- and 120-minute time points relative to mice that received lorcaserin and oxycodone (#*P* < 0.01, 2-way ANOVA with multiple comparisons). Each point is represented as the mean ± S.E.M and by at least 9 mice.



** P-value <0.001
One-way ANOVA

Figure 2.11: Subcutaneous lorcaserin attenuated exploratory activity in mice. Lorcaserin (1 and 2 mg/kg, s.c.) significantly attenuated motor activity relative to control mice (P < 0.001, one-way ANOVA). The low dose of lorcaserin (0.5 mg/kg, s.c.) was not significantly different from controls. A minimum of five mice were used per group and bars are represented as the mean ambulatory counts in the total 40-minute testing period ± S.E.M.

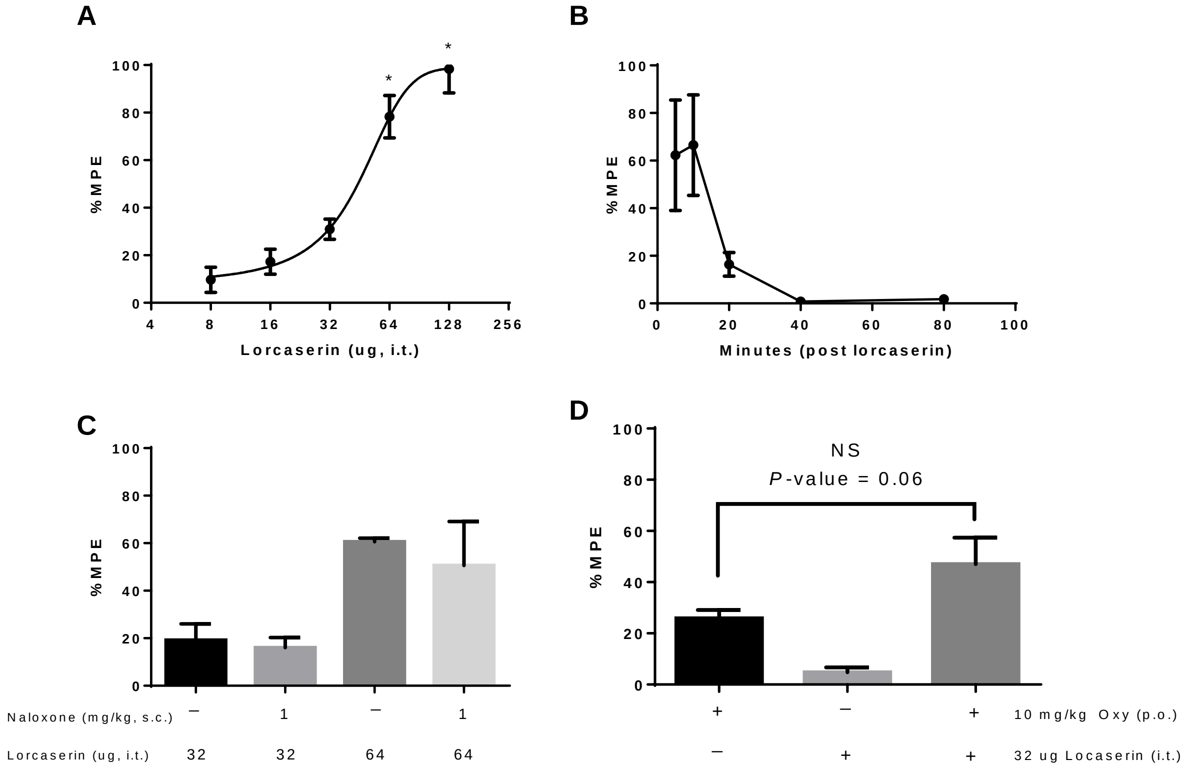


Figure 2.12: Intrathecal lorcaserin produced dose-dependent antinociception that was not blocked by administration of naloxone. Intrathecal lorcaserin (A) dose-dependently produced an antinociceptive response, with a significant effect occurring at 64 µg and peak effects at 10 minutes (B). The effect of 64 µg lorcaserin (i.t.) was not blocked by naloxone (1 mg/kg, s.c., figure C). A subthreshold dose of 32 µg lorcaserin (i.t.) (D) did not produce a significant additive effect on a subthreshold dose of oral oxycodone. Each data point is represented by a minimum of five mice and presented as the mean ± S.E.M and separate cohorts of mice were used for each experiment. Values were compared using a one-way ANOVA.

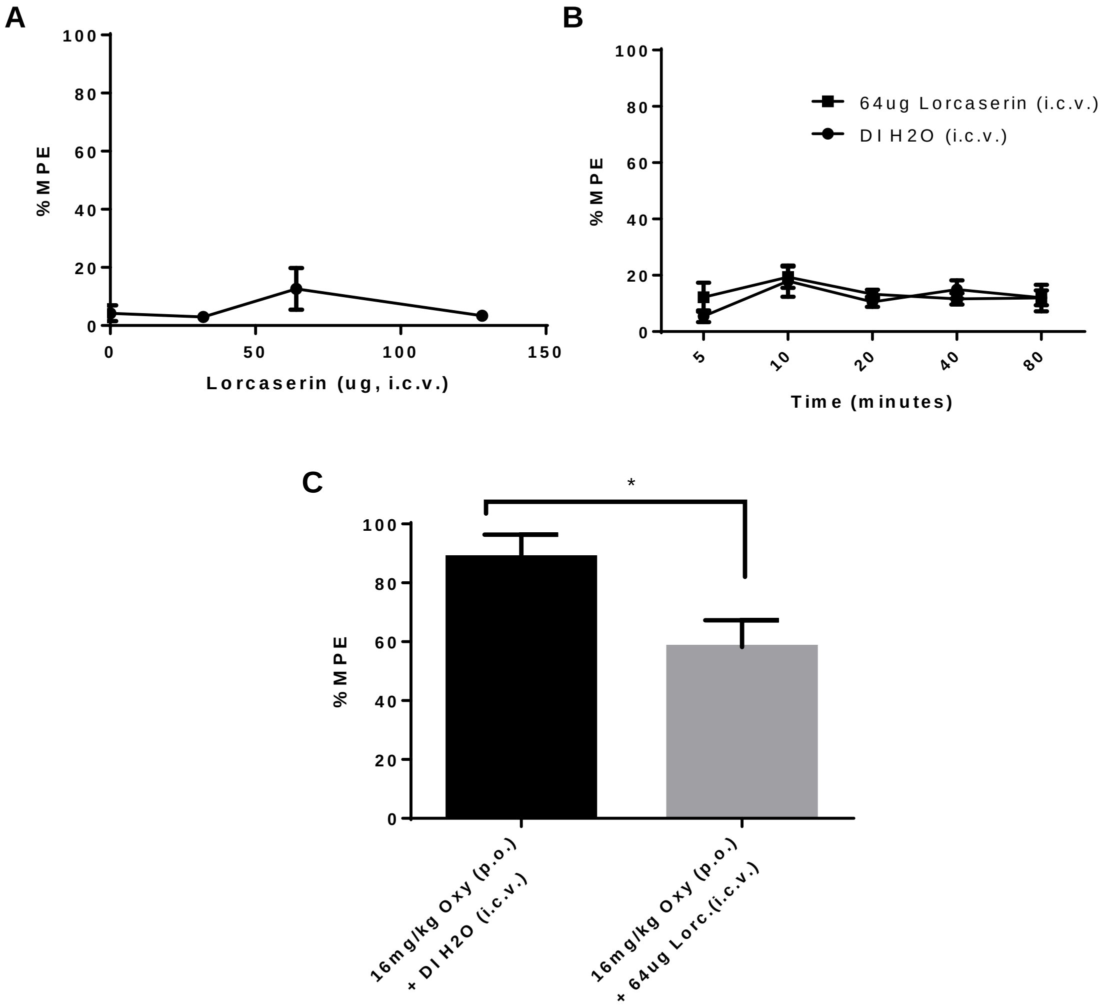


Figure 2.13: Intracerebroventricular (i.c.v.) lorcaserin did not produce antinociceptive effects on its own and attenuated the antinociceptive effect of oxycodone. The administration of intracerebroventricular lorcaserin, at all doses tested up to 128 µg, did not produce antinociceptive effects (A). The time course of locaserin (64 µg, i.c.v.) was inactive at all time points tested (B). An inactive dose of lorcaserin (64 µg, i.c.v.) (C) significantly attenuated the antinociceptive effect of oral oxycodone (*P<0.05, one-way ANOVA). Antinociceptive activity was assessed using the warm-water tail withdrawal assay. All values are represented by at least four to ten mice per group and the mean ± SEM.

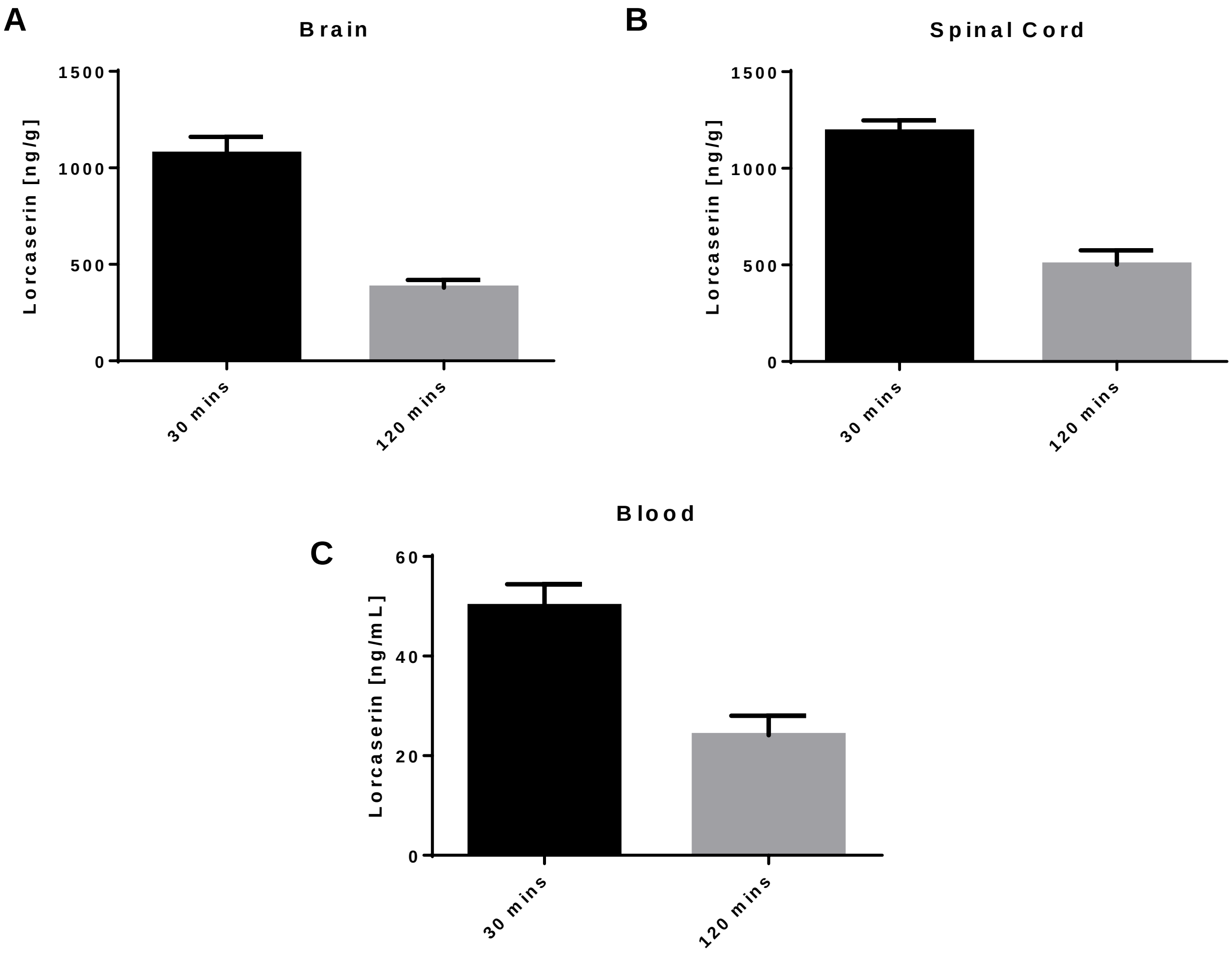


Figure 2.14: Blood, brain, and spinal cord distributions of subcutaneously administered lorcaserin. Lorcaserin (2 mg/kg, s.c.) preferentially accumulated in the central tissues relative to blood at both 30 and 120 minutes post administration. Subcutaneous lorcaserin displayed 20-fold greater accumulation in the brain relative the blood. Five to ten mice were used for each group tested and, with the bars representing the mean ± S.E.M.

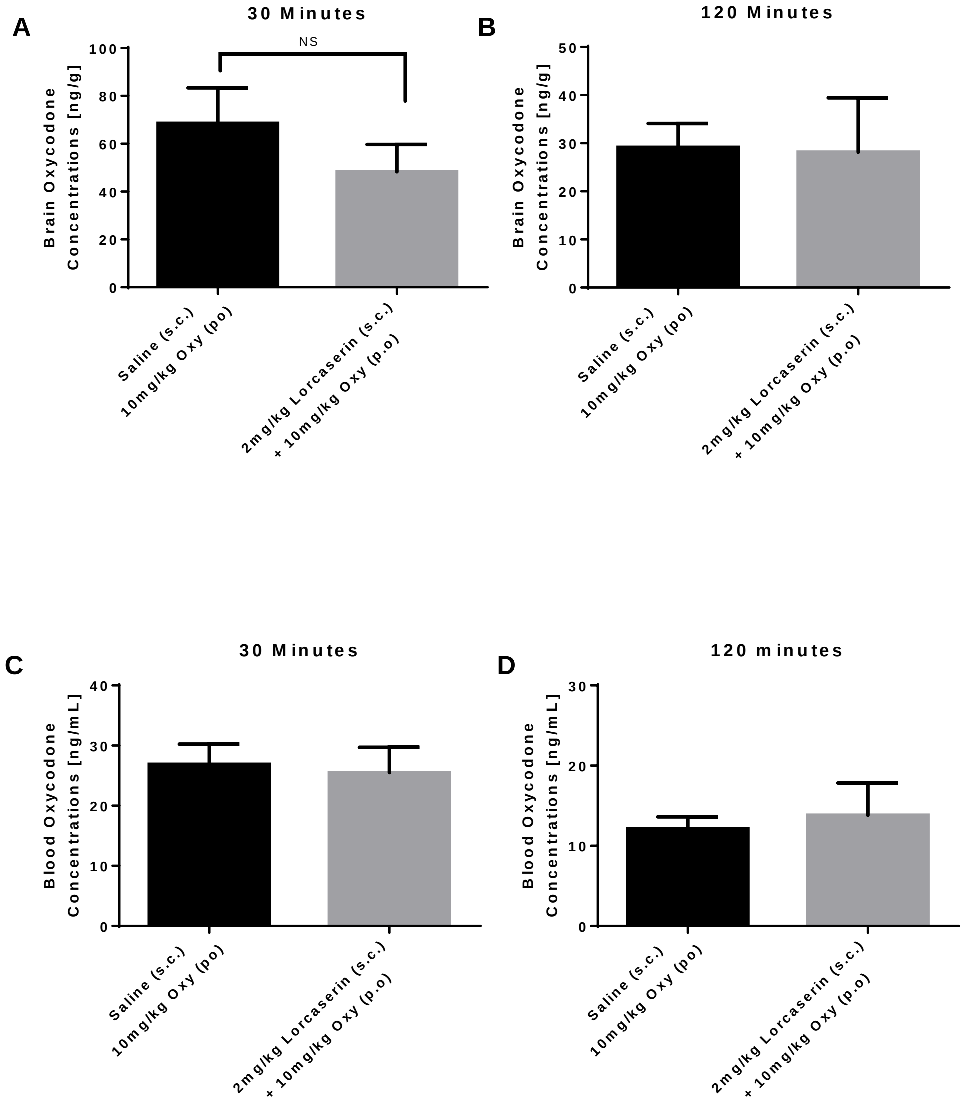


Fig. 2.15: Subcutaneous lorcaserin did not significantly alter the distribution of oral oxycodone in the brain or the blood. Mice were injected with lorcaserin (2 mg/kg s.c.) and then gavaged thirty minutes later with an ED50 dose of oral oxycodone (10 mg/kg, p.o.). Blood and brain samples were collected at the time points where lorcaserin significantly altered the time course of oxycodone’s antinociceptive effect. Lorcaserin pretreatment did not significantly alter the kinetic distribution of oxycodone at either time points tested. Values were compared

using the Student's unpaired two-tailed *t*-test with $P > 0.05$. A minimum of eight mice were used per group and the values are displayed as the mean ± S.E.M.

V. Discussion

In spite of the current opioid epidemic, opioids are still commonly used for the treatment of moderate to severe pain, but there is renewed interest in developing opioid-sparing treatments that reduce the overall quantity of opioid consumed (Sullivan *et al.*, 2016; F Collins *et al.*, 2017; Volkow and Collins, 2017). Although current opioid-sparing drugs, such as NSAIDs and antidepressants, are demonstrably well-regarded and tolerated, each drug class presents its own set of risks and alternatives need to be investigated (Watson, 2000; Saarto and Wiffen, 2007; Huang *et al.*, 2008; Derry *et al.*, 2009, 2013; Gilron, 2016). Lorcaserin is a 5-HT$_{2C}$ receptor agonist that is FDA approved for the treatment of obesity and is under investigation as a possible novel therapeutic agent for treating addiction (Bubar and Cunningham, 2008; Smith *et al.*, 2009, 2010; Fidler *et al.*, 2011; CDER and FDA, 2016; GT Collins *et al.*, 2017). Preclinical studies have demonstrated that lorcaserin suppressed the abuse-related effects of opioids and reduced naloxone-precipitated withdrawal symptom severity in mice (Wu et al., 2015; Zhang et al., 2015; Neelakantan et al., 2017). Based on the observations that lorcaserin can alter one aspect of the pharmacological effects of opioids, the purpose of these studies was to evaluate the effect of lorcaserin on another important component of opioid evaluation: opioid-induced antinociception. The critical findings of these studies suggest the use of lorcaserin as a potential opioid-sparing adjunct for the treatment of pain which necessitates further investigation. The goal of the studies represented here was to evaluate the effect of lorcaserin on acute opioid antinociception in a model of acute pain.

Dose-response analysis of subcutaneous lorcaserin on opioid antinociception. Initial studies were conducted to evaluate the dose-responsive effect of lorcaserin on the antinociceptive properties of several clinically used opioids, such as oxycodone, morphine, fentanyl and methadone. The most important finding of these studies was that lorcaserin, and another 5-HT_{2C} receptor agonist WAY163909, produced increases in both the potency and the time course of activity of oral oxycodone and similar opiates, including morphine and fentanyl. These combined effects of increased potency and increased duration of action are desirable traits of an opioid-sparing adjunctive therapy, as they will reduce the dose and frequency of opioid needed to treat pain.

Dose-response analysis of lorcaserin and oxycodone revealed that lorcaserin produced relative shifts in the ED_{50} values of oxycodone, morphine, fentanyl (but not methadone which will be discussed further). As the dose of lorcaserin increased, the relative opioid ED_{50} of oxycodone decreased and significant shifts were observed at 2 mg/kg of lorcaserin with a resulting ED_{50} of ~ 4 mg/kg for oxycodone. It is important to note that the effect of lorcaserin on the ED_{50} value of oxycodone produced a biphasic curve, where lower doses of lorcaserin (2 mg/kg) produced a greater shift in the ED_{50} than higher doses of lorcaserin (4 mg/kg). It is possible that this may be due to off-target effects of lorcaserin that may be mediated through the 5-HT_{2A} receptor because lorcaserin has some affinity and moderate efficacy at the 5-HT_{2A} receptor (Thomsen *et al.*, 2008).

Interestingly, this enhancement of opioid antinociception by lorcaserin was not antagonized by the administration of naloxone, suggesting that the observed effects are not mediated through the opioid system and lorcaserin has a distinct mechanism of action. The antinociceptive effects of 5-HT_{2C} receptor agonists are suggested to be partially mediated through changes in noradrenergic and cholinergic signaling and this may be a possible mechanism for this interaction (Obata *et al.*, 2007).

In order to determine if the effect of lorcaserin was due to some other effect than 5-HT_{2C} receptor activation, another non-structurally related 5-HT_{2C} agonist was evaluated. WAY 163909 is a selective 5-HT$_{2C}$ receptor agonist and a 5-HT$_{2A}$ receptor antagonist (Dunlop *et al.*, 2005). Similar to lorcaserin, treatment with another 5-HT$_{2C}$ agonist, WAY 163909, enhanced oxycodone antinociception and produced its greatest enhancement at the lower tested dose (1 mg/kg). The lack of dose-responsivity of 5-HT$_{2C}$ agonists in their effects on opioids may be intrinsic to this class of compound and mediated through some unknown off-target effect. Based on the concurrence of their effects, however, it is clear that activation of the 5-HT$_{2C}$ receptor is an important component of the earlier described enhanced opioidergic effects.

Unlike previously tested opioids, the antinociceptive potency of methadone was not altered by lorcaserin at any of doses tested. Methadone is used as both an agonist-replacement therapy and as a treatment for chronic pain (Brown *et al.*, 2004). Methadone has a complex pharmacological profile that includes serotonergic reuptake inhibition and is a much higher efficacy agonist relative to traditional opioids like morphine or oxycodone (Horng *et al.*, 1976; Codd *et al.*, 1995; Ebert *et al.*, 1995; Brown *et al.*, 2004; Callahan *et al.*, 2004). The lack of potentiation by lorcaserin could be due to a ceiling effect associated with methadone’s serotonergic activity or that intrinsic efficacy of an opioid agonist is an important determinant when in combination with lorcaserin. Opioid agonist efficacy has been reported as an important determinant in drug-drug interactions, specifically in combination with SSRIs and TCAs (Gatch *et al.*, 1998; Banks *et al.*, 2010).

Opioid efficacy is proposed to be an important factor in the mechanisms that regulate MOR-trafficking (Keith *et al.*, 1996, 1998; Sternini *et al.*, 1996; Whistler and von Zastrow, 1998; Bohn *et al.*, 2004; McPherson *et al.*, 2010). High efficacy agonists, such as methadone and DAMGO, are reported to produce robust receptor internalization and in contrast, relatively lower efficacy agonists, such as morphine, have preferentially induced a desensitized receptor state without significant receptor endocytosis (Keith *et al.*, 1996, 1998; Sternini *et al.*, 1996; Whistler and von Zastrow, 1998; Borgland *et al.*, 2003; Arttamangkul *et al.*, 2008; McPherson *et al.*, 2010). It is possible that the effect of lorcaserin on opioid antinociception may be due to heterologous alterations in receptor trafficking, as activation of the 5-HT$_{2C}$ receptor converges onto protein kinase C signaling which is known to be an important regulator MOR desensitization (Laura M. Bohn *et al.*, 2000; Kohout *et al.*, 2003; Bailey *et al.*, 2004; Gabra *et al.*, 2008; Hull *et al.*, 2010; Jacob *et al.*, 2018). Further study evaluating MOR trafficking and lorcaserin treatment interactions are necessary though.

Deletion of the 5-HT$_{2A}$ receptor and the effect of lorcaserin. Lorcaserin has notable affinity as a partial agonist for the 5-HT$_{2A}$ receptor (Thomsen *et al.*, 2008). Although the literature supports a pro-nociceptive role for the 5-HT$_{2A}$ receptor, we evaluated its contributions in the enhanced opioid antinociceptive effect (by lorcaserin) using a global 5-HT$_{2A}$ KO model (Tokunaga *et al.*, 1998; Zhang *et al.*, 2001; Nitanda *et al.*, 2005; Wei *et al.*, 2005; Nakajima *et al.*, 2008; Huang *et al.*, 2009, 2011; Lippold and Dewey, 2017). Compared to the wildtype controls, genetic deletion of the 5-HT$_{2A}$ receptor trended towards increased opioid potency but failed to reach statistical significance. In addition, the effect of lorcaserin on opioid antinociception in these mice also did not significantly shift the ED$_{50}$. Therefore, the role of the 5-HT$_{2A}$ receptor in the effects elicited by lorcaserin is unclear and requires further study.

It should also be noted that co-activation of the 5-HT$_{2A}$ receptor and the MOR is reported to produce measurable changes in MOR trafficking (Lopez-Gimenez *et al.*, 2008). Treatment with a 5-HT$_{2A}$ antagonist is reported to block desensitization of the MOR and may underlie the trend towards increased morphine potency that was observed in the 5-HT$_{2A}$ knockout model.

Additional studies have reported that the 5-HT$_{2A}$ receptor is an important component of fenfluramine's (a serotonin releaser) enhancement of the antinociceptive effect of morphine in monkeys and furthermore, addition of a 5-HT$_{2A}$ agonist potentiated the antinociceptive effect of morphine on its own (Li *et al.*, 2011). In contrast, a similar study in rodents show that treatment with a 5-HT$_{2A}$ agonist only altered the antinociceptive activity of morphine at doses that had a modest antinociceptive effect on their own (Li *et al.*, 2013). There are clear differences in 5-HT$_{2A}$ expression between species, though the functional effect of these species-differences has yet to be fully elucidated (Juan F. López-Giménez *et al.*, 1997; López-Giménez *et al.*, 1998).

Lorcaserin increased the time course of oxycodone. In addition to characterizing the dose-related effects of lorcaserin on opioid antinociception, it was important to characterize the effect on lorcaserin on opioid time course of activity. Two doses of lorcaserin were tested, a dose that produced a significant shift in the ED$_{50}$ of oxycodone (2 mg/kg, s.c.) and a subthreshold dose (0.5 mg/kg, s.c.) that shifted the dose-response curve but not to a significant degree. The 2 mg/kg dose of lorcaserin significantly extended the time course of oxycodone up to 2 hours post a single administration. The low dose (0.5 mg/kg s.c.) produced a modest, but statistically significant, enhancement of oxycodone's activity at the 60-minute time point. In addition, the enhanced time course was blocked by treatment with the selective 5-HT$_{2C}$ receptor antagonist, SB242084, and supports the notion that activation of the 5-HT$_{2C}$ receptor is responsible for the enhanced opioid antinociceptive responses.

A potential confound that is inherent to assays that necessitate motor responses is that the observed analgesic response is due to motor impairment of the withdrawal response (Le Bars *et al.*, 2001). Given this, lorcaserin's effects on motor activity were evaluated and the data show that lorcaserin produced dose-dependent reductions in motor activity. The data suggest that lorcaserin is more potent to suppress motor function than it is to significantly alter the acute antinociceptive effects of opioids. It is important to note, however, that in the studies conducted to evaluate the effect of lorcaserin on motor activity, the 2 mg/kg dose significantly attenuated motor activity while the 0.5 mg/kg dose did not. The effect of the low dose of lorcaserin (0.5 mg/kg) in the antinociceptive time course of oxycodone at the 60-minute mark suggested that the observed antinociceptive effects may not be due to changes in motor activity, as this was not a dose that significantly altered motor function. It should also be noted that lorcaserin on its own was inactive in the warm-water tail withdrawal test despite its effects in the locomotor assay.

The differential site-dependent effects of lorcaserin on opioid antinociception: intrathecal versus intracerebroventricular actions. Anatomical locus of activity is important for determining the site of action through which a drug is producing its effects. While these studies may not directly provide insight into the opioid-sparing antinociceptive properties of lorcaserin, these studies do provide additional data on the role of the 5-HT$_{2C}$ receptors in spinal and supraspinal sites and how activation of these receptors alter opioid antinociception.

Previous studies have almost exclusively evaluated the antinociceptive effects of 5-HT$_{2C}$ receptor agonists through intrathecal administration in rodent models of trigeminal neuralgia and neuropathy (Obata *et al.*, 2004; Nakai *et al.*, 2010; Ogino *et al.*, 2013). In light of these publications, it was not unexpected that intrathecal administration of lorcaserin, a 5-HT$_{2C}$ receptor agonist with greater selectivity, produced a similar dose-dependent antinociceptive response in the model of acute pain. The lack of antagonism by naloxone in blocking this antinociceptive effect suggested that the spinal effect of lorcaserin is not mediated through opioid-dependent mechanisms. Earlier studies evaluating the intrathecal activity of 5-HT$_{2C}$ receptor agonists (as a caveat, these agonists did not possess improved selectivity for the 5-HT$_{2C}$ receptor and may have had off-target effects) suggest that indirect interactions may be occurring through spinal noradrenergic or cholinergic mechanisms (Obata *et al.*, 2007). Indirect interactions with the noradrenergic system could be one mechanism that lorcaserin could be affecting to produce its effects through with activation of the 5-HT$_{2C}$ receptor. Overall, these data support the hypothesis that the antinociceptive effect of intrathecal lorcaserin are due to its action on 5-HT$_{2C}$ receptors in the spinal cord and not due to an effect on the endogenous opioid systems.

Surprisingly, intracerebroventricular administration of lorcaserin was completely inactive at all doses tested in the warm-water tail withdrawal test. In spite of evidence that compounds that are administer intracerebroventricularly can be rapidly transported to the lowest portions of the spinal cord (where lorcaserin was active), i.c.v. administration of lorcaserin did not have a significant antinociceptive effect (Ohlsson *et al.*, 1982). The time course of i.c.v. lorcaserin revealed a similar result that it was inactive up to 80 minutes post i.c.v. administration. In spite of the potential for circulation into the spinal cord following i.c.v. administration, it is clear that possibly sufficient concentrations of lorcaserin have not circulated into the spinal cord to produce a significant effect and that intracerebroventricular and intrathecal administrations of lorcaserin have distinctly different antinociceptive effects.

Following the idea that central and spinal 5-HT$_{2C}$ receptors serve differential roles, reports suggest that the 5-HT$_{2C}$ receptors in the brain may serve a “pro-nociceptive” role, where genetic knockdown or antagonism of the 5-HT$_{2C}$ receptor improves the antinociceptive efficacy of SSRIs in preclinical neuropathic pain models (Grégoire and Neugebauer, 2013; Ji *et al.*, 2017). The site-specific role of 5-HT$_{2C}$ receptors has been demonstrated once before, where administration of the 5-HT$_{2C}$ receptor antagonist, SB242084, into the basolateral amygdala (BLA) augments the antinociceptive properties of an SSRI in a rodent model of arthritis (Grégoire and Neugebauer, 2013). Furthermore, genetic knockdown of the 5-HT_{2C} receptor in the amygdala inhibited the elicitation of neuropathic pain-related behaviors in a model of spinal nerve ligation (Ji *et al.*, 2017). Taken together, our studies agree with the notion that 5-HT_{2C} receptors centralized within the brain serve a differential role in pain that remains to be elucidated. Although these studies do not directly provide evidence of the opioid-sparing role of lorcaserin, they provide insight into the role of the 5-HT_{2C} receptor in pain-states and provide information that may be useful in the development of other serotonergic compounds.

The effect of intrathecal and intracerebroventricular administrations of lorcaserin on oral oxycodone-induced antinociception are similarly different and suggest that the primary site of lorcaserin's actions on opioid antinociception are mediated through the spinal cord. The site of action was evaluated by administering an ED_{50} dose of oxycodone (10 mg/kg, p.o.) in combination with a subthreshold dose of IT lorcaserin (32 μg). Although the combination did not produce a significant effect (*P*-value = 0.0692), the measured antinociceptive response roughly doubled. The attenuation of oxycodone's effect by ICV lorcaserin could be due to changes in body temperature. 5-HT_{2C} receptor agonists are shown to be thermogenic and increase body temperature (Hayashi *et al.*, 2004). The warm-water tail withdrawal test is sensitive to changes in body temperature and typically, as body temperature increases, the animal's latency to withdraw its tail from the water inversely decreases (Tjolsen and Hole, 1993; Le Bars *et al.*, 2001). Alternatively, it could be due to the discussed "pro-nociceptive" role of brain-centralized 5-HT_{2C} receptors. Nonetheless, the observation that activation of spinal and supraspinal 5-HT_{2C} receptors have such vastly differential effects remains to be further investigated.

Brain, Spinal Cord, and Blood concentrations of Lorcaserin. Based on the earlier nociceptive data involving the site-specific effects of lorcaserin on opioid antinociception, further studies were conducted to evaluate the distribution of lorcaserin in the central tissues relative to the blood. The results revealed considerable accumulation of lorcaserin in the central tissues, by ~20-fold greater concentrations, than in the blood. This observation is in agreement with previous reports in preclinical studies, using rodents and monkeys, reporting similar accumulation of lorcaserin in the brain relative to blood plasma (CHMP, 2013). The accumulation of lorcaserin in central tissues is shown to not be due to P-glycoprotein (P-gp) activity, as lorcaserin is not a substrate of the P-gp transporter but simply a highly soluble and highly permeable compound (Center for Drug Evaluation and Research, 2012). It is important to note that the preferential CNS-accumulation of lorcaserin was not observed during clinical trials in humans, whereby measured lorcaserin concentrations were greater in human plasma relative to cerebrospinal fluid (Center for Drug Evaluation and Research, 2012). Although the unusual accumulation of lorcaserin in specific tissues is compelling, it does not appear to be clinically relevant.

Central nervous system and Blood Concentrations of Oxycodone after Lorcaserin treatment.

Lorcaserin is a competitive inhibitor of CYP P450 enzymes, particularly CYP2D6, and is a mild inducer of CYP3A4 and thus displayed potential for drug-drug interactions (Center for Drug Evaluation and Research, 2012). Opioids are subjected to phase 1 metabolism by CYP enzymes and oxycodone, in particular, is subjected to metabolism primarily by CYP3A4 and to a lesser extent by CYP2D6 (Lalovic *et al.*, 2004; Smith, 2009; Samer *et al.*, 2010; Söderberg Löfdal *et al.*, 2013; Stamer *et al.*, 2013). The potential for lorcaserin and oxycodone interactions were of concern because increases in oxycodone concentrations could be lethal. The oxycodone concentrations were measured to ensure that the potentiating effects of lorcaserin were not mediated through inhibition of oxycodone metabolic pathways. Blood, brain and spinal cord concentrations of oxycodone were evaluated following a pretreatment with lorcaserin (2 mg/kg, s.c.) and a single gavage of oxycodone (10 mg/kg, p.o.). As discussed earlier, the time-course of oxycodone's antinociceptive activity was significantly enhanced and extended and this could be attributed to changes in metabolism. Following analysis using UPLC-MS/MS, no significant changes in blood, brain or spinal cord concentrations with lorcaserin treatment were observed. Although lorcaserin does present the potential for drug-drug metabolic interactions, this effect did not underlie the behavioral effects observed.

Final Conclusions

The primary goal of these studies was to investigate the effect of lorcaserin on opioid antinociception and to determine the general mechanism through which these effects are occurring. The antinociceptive effect of lorcaserin alone was investigated and determined to produce dose-dependent antinociception when administered intrathecally but not when given by any other route. This effect was also not blocked by the opioid antagonist, naloxone. We hypothesize that this effect was due to its selectivity for the 5-HT$_{2C}$ receptor in the spinal cord. The studies demonstrate that parenterally-administered lorcaserin significantly enhanced the acute effects and the time course of opioids and that this effect is primarily mediated through spinal serotonergic receptors. Therefore, we suggest that 5-HT$_{2C}$ receptor agonists, such as lorcaserin, deserve additional investigation into their potential use as opioid sparing agents.

The significance of these data are that this is the first demonstration of the effect of 5-HT$_{2C}$ agonists altering the antinociceptive effects of opioids. Previously, studies have only investigated the effect of lorcaserin (and similar agonists) on the addictive and dependence-related properties of opioids. In many cases, opioids are typically consumed for the treatment of pain and it was critical to evaluate the interactions between these two compounds in an acute dosing fashion before moving onto evaluations of tolerance interactions. These studies, in conjunction with previously published work, suggest that lorcaserin and oxycodone may be a useful combination in that it increases the favorable antinociceptive properties while reducing the abuse-related properties. Another finding from this work is that overall, the data suggest that the dose of opioid that is necessary to treat pain may be reduced, thus additionally reducing the risk to the patient.

Chapter 3

The Effect of Lorcaserin on the Development of Opioid Tolerance

The data for the electrophysiology studies was the result of a wonderful collaboration with Dr. Jacy Jacob of the Department of Pharmacology & Toxicology at VCU. She conducted the experiments and collected the data for the studies described in Figures 3.3 and 3.4.

I. Summary

Oxycodone and lorcaserin produce antinociception through activation of mu opioid receptors and 5-HT$_{2C}$ receptors in the central nervous system, with notable effects mediated through the spinal cord. Chronic treatment with oxycodone results in many unwanted side-effects that can be mitigated by the addition of an opioid-sparing adjunct, such as lorcaserin, that reduces the dose of opioid needed. The goal of these studies was to evaluate the effect of lorcaserin on the development of acute and multiple day tolerance models *in vivo* and *in vitro*. In the whole animal studies utilizing the warm-water tail withdrawal assay, lorcaserin differentially modulated the development of acute and multiple-day tolerance. Lorcaserin significantly blocked the development of acute tolerance but only partially attenuated the development of multiple-day tolerance. Acute tolerance was further assessed on a single cell level using electrophysiological recording methods in dorsal root ganglion neurons and the results showed that overnight co-incubation with lorcaserin and oxycodone significantly attenuated the development of tolerance. Agonist-stimulated [^{35}S]GTPγS binding was used to assess mu opioid receptor activity after the multiple-day treatment paradigm with oxycodone and lorcaserin. Chronic administration of oxycodone decreased MOR-stimulated [35S]GTPyS binding in the spinal cord and reduced basal activity of the receptors. Treatment with lorcaserin partially restored basal activity but did not significantly alter maximal stimulation of G-protein activity relative to chronic oxycodone

treatment. These results demonstrate that lorcaserin has differential effects on opioid tolerance that depend on the frequency of administration and that the mechanisms underlying acute and multiple-day tolerance are distinct. Furthermore, these data suggest that combination treatment with lorcaserin may be a potential opioid-sparing alternative that requires further investigation.

II. Rationale

Tolerance to the analgesic effects of opioids is a clinically relevant effect that may require dose-escalation of the opioids and opioid-switching (Mehta and Langford, 2006; Huxtable et al., 2011; Simpson and Jackson, 2017). Following prolonged opioid exposure, tolerance is thought to develop in two phases: an acute component and a chronic component (Cox et al., 1968; Rosenfeld et al., 1977; Huidobro-Toro and Way, 1978; Fairbanks and Wilcox, 1997; Bohn et al., 2000; Williams et al., 2013). Based on this idea of two distinct phases of tolerance, we completed a thorough evaluation of the effect of lorcaserin on opioid tolerance. Acute opioid tolerance was assessed both in vivo and in vitro. Antinociceptive tolerance was induced in a manner that is similar to previously published reports and used a model of exposure that involved a single bolus or limited exposure to 24 hours (Cox et al., 1968; Huidobro-Toro and Way, 1978; Ling et al., 1989; Fairbanks and Wilcox, 1997; Bohn et al., 2000). Based on the observation that lorcaserin completely attenuated the development of acute tolerance in vivo, further studies were conducted to evaluate the effect of lorcaserin on a single cell level in dorsal root ganglion neurons. DRGs are important components in the transmission of nociceptive information and are implicated as an important component in the development of opioid tolerance (Corder et al., 2017; Jacob et al., 2018). Following evaluation of lorcaserin's effects on the acute component of opioid tolerance, further studies were conducted to evaluate the effect of lorcaserin on the chronic component of tolerance both in vivo and in vitro. The chronic component of opioid exposure is marked by differential rates of tolerance development (Shook et al., 1987; Ling et al., 1989; White and Irvine, 1999; Ross et al., 2008; Hill et al., 2016). Therefore, the effect of lorcaserin was evaluated on two subcomponents of chronic opioid exposure: antinociceptive tolerance and opioid-induced constipation. The purpose of the constipation study was to conduct a thorough evaluation of the effect of lorcaserin on several aspects associated with chronic opioid administration. Lorcaserin treatment did not alter the constipating effect of oxycodone but did partially attenuate the development of antinociceptive tolerance. Based upon this observation, we developed the hypothesis that lorcaserin is altering the functional activity of the MOR and this change underlies the observed in vivo effect in the chronic oxycodone model. Changes in MOR-mediated signaling occurs following chronic administration of an opioid in vivo and this alteration in functionality can be modified by the addition of non-opioid ligands (Tao et al., 1993; Sim et al., 1996; Smith et al., 2007; Sim-Selley et al., 2009). These studies were conducted using [^{35}S]GTPγS binding because it assesses the initial stage of receptor-mediated signaling following opioid exposure.

III. Introduction

Opioid tolerance is a complex phenomenon that is thought to be comprised of many stages and distinct mechanisms. Tolerance is a physiological adaptation that follows acute or repeated administrations of a drug such that increased doses of a drug are required to produce pharmacological effects that were previously elicited by smaller doses; this effect is characterized by a rightward shift of the dose-response curve (Brunton *et al.*, 2011; Savage *et al.*, 2003). There are many mechanisms and levels through which tolerance manifests itself, including: changes in behavior, adaptations in drug metabolism, alterations in receptor signaling on a cellular level (receptor desensitization and downregulation), and compensatory changes in intracellular signaling (Williams *et al.*, 2001, 2013; Brunton *et al.*, 2011; Cahill *et al.*, 2016). Several hallmark opioid effects, such as antinociception, respiratory depression, and constipation, are independently marked by differences in the mechanisms through which tolerance occurs and their rates of development (Shook *et al.*, 1987; Ling *et al.*, 1989; Ross *et al.*, 2008; Hill *et al.*, 2016; Jacob *et al.*, 2017).

The mechanisms through which tolerance develops has been divided into two stages: short-term (or acute) tolerance and long-term (chronic) tolerance (Cox *et al.*, 1968; Rosenfeld *et al.*, 1977; Huidobro-Toro and Way, 1978; Fairbanks and Wilcox, 1997; Laura M. Bohn *et al.*, 2000; Williams *et al.*, 2013). The many methods that have been used to produce what investigators term "short" or 'long-term" tolerance varies so much from one study to another, one should define the terms for each specific definition. For the purpose of these studies, short-term (acute) tolerance is defined as the tolerance that develops within the time course of one day and is mediated through rapid changes in phosphorylation, desensitization, and endocytosis of the receptor (Cox *et al.*, 1968; Sibley *et al.*, 1984, 1987; Hausdorff *et al.*, 1989; Kovoor *et al.*, 1998; Lefkowitz, 1998; Whistler and von Zastrow, 1998; Laura M. Bohn *et al.*, 2000; Borgland *et al.*, 2003; Bohn *et al.*, 2004). Short-term tolerance is often considered to be the initiation phase preceding long-term tolerance (Rosenfeld *et al.*, 1977; Fairbanks and Wilcox, 1997). Long-term tolerance in this study is defined as the tolerance that develops after a period of several days to weeks and is presumed to involved multiple regulatory and compensatory mechanisms, such as receptor downregulation and changes in receptor constitutive activity (Tempel and Zukin, 1987; Tempel, 1991; Tao *et al.*, 1993; Z Wang *et al.*, 1994; Wang *et al.*, 2004; Sim-Selley, 2005; Shoblock and Maidment, 2006; Sim-Selley *et al.*, 2009). Based on these principles, the studies described herein employed two models of tolerance. The short-term tolerance model was based off of early studies that administered a large single dose of opioid and challenged the following day (Cox *et al.*, 1968; Huidobro-Toro and

Way, 1978; Wigdor and Wilcox, 1987; Fairbanks and Wilcox, 1997). Long-term tolerance models vary across studies but for these studies, we opted to use a 4 day treatment paradigm whereby doses of oxycodone were administered orally, twice a day for four days (Jacob *et al.*, 2017).

The progression of opioid tolerance can be modulated by activation of other receptors, such as cannabinoid, N-methyl-D-aspartate, dopamine receptors or serotonin receptors (Ho *et al.*, 1975; Larson and Takemori, 1977; Siu-Chun *et al.*, 1996; Smith *et al.*, 2007; Lopez-Gimenez *et al.*, 2008; Song *et al.*, 2015; Dai *et al.*, 2016). Modulation of the serotonergic system has emerged as a target for altering the abuse-related properties and general pharmacological effects of opioids. In fact, co-activation of the serotonin type-2A receptor (5-HT$_{2A}$) and the MOR results in changes in MOR receptor trafficking that are heavily implicated in the development in opioid tolerance (Lopez-Gimenez *et al.*, 2008). In many ways, the 5-HT$_{2A}$ receptor and the 5-HT$_{2C}$ receptor function in an inverse manner and further studies evaluating the effect of a 5-HT$_{2C}$ agonist on the chronic effects of opioids is necessary (Abbott *et al.*, 1996; Tokunaga *et al.*, 1998; Willins and Meltzer, 1998; Porras *et al.*, 2002; Obata *et al.*, 2004; Bortolozzi *et al.*, 2005; Nakai *et al.*, 2010; Ogino *et al.*, 2013).

Several studies have described the effect of 5-HT$_{2C}$ receptor agonists, such as lorcaserin, in preclinical models of pain. Overall, 5-HT$_{2C}$ receptor agonists behave as antinociceptive agents in models of trigeminal neuropathy, fibromyalgia, and chronic constriction injuries (Obata *et al.*, 2003, 2004, 2007; Nakai *et al.*, 2010; Ogino *et al.*, 2013). Two studies have specifically investigated the effect of lorcaserin on opioid pharmacology. First, lorcaserin is shown to attenuate the abuse-related effects of oxycodone in a rodent self-administration model (Neelakantan *et al.*, 2017). Second, lorcaserin inhibits the induction and expression of behavioral sensitization in mice treated chronically with morphine or heroin (Wu *et al.*, 2015; Zhang *et al.*, 2015). Those same studies also demonstrate that lorcaserin significantly ameliorates naloxone precipitated withdrawal behaviors in morphine- and heroin-dependent mice.

Work from our lab (chapter 2 of this dissertation) shows that lorcaserin also alters the acute antinociceptive effects of oxycodone and similar opiates, such as morphine and fentanyl. Although previous studies have evaluated the effect of lorcaserin on naloxone-precipitated opioid withdrawal, little is known about its effect on opioid tolerance (Wu *et al.*, 2015; Zhang *et al.*, 2015). Evaluating these interactions are important for several reasons. Dose escalation of opioids can exacerbate opioid-induced hyperalgesia and overall, increase the risk of mortality for the patient (Dasgupta *et al.*, 2015). Understanding alternative mechanisms through which opioid tolerance can be favorably modulated for the patient is of critical importance in light of our current opioid epidemic. Therefore, the aim of these studies was to characterize the effect of lorcaserin, a 5-HT_{2C} receptor agonist, in models of opioid tolerance and to elucidate the mechanism through which these effects may be occurring.

IV. Methods

Drugs and Chemicals

Dulbecco's modified Eagle medium (DMEM), Hank's balanced salt solution (HBSS) and fetal bovine serum were purchased from Gibco (Grand Island, NY). Papain was purchased from Worthington Biochemical Corporation (Lakewood, NJ). B27 supplement, L-glutamate, and pencillin/streptomycin were purchased from Invitrogen (Carlsbad, CA). Glial cell line-derived neurotrophic factor (GDNF) was purchased from Neuromics (Edina, MN). Glass cover slips were purchased from ThermoFisher Scientific (Waltham, MA). Laminin was purchased from BD Biosciences (San Jose, CA) and poly-D-lysine was purchased from MP Biomedicals (Solon, OH). 24-well cell culture dishes were purchased from CELLTREAT (Pepperell, MA). Collagenase from

Clostridium histolyticum, magnesium chloride (MgCl2), calcium chloride (CaCl2), NaCl, KCl, HEPES, EGTA, sodium dihydrogen phosphate (NaH2PO4), glucose, ATP disodium salt, K-aspartic acid, potassium hydroxide (KOH) and sodium hydroxide (NaOH) were purchased from Sigma Aldrich (St. Louis, MO). Oxycodone HCl was obtained from the National Institutes of Health National Institute on Drug Abuse (Bethesda, MD) and dissolved in ddH$_2$0. Lorcaserin HCL was purchased from Cayman Chemical (Ann Arbor, MI). [35S]GTPyS (1250 Ci/mmol) was purchased from PerkinElmer.

Animals. For the in-vivo, tolerance experiments, adult male Swiss Webster mice (25-35g) and at least 7 weeks of age were purchased from ENVIGO (Frederick, MD). For the electrophysiology experiments: adult male C57/BL6, 25-30g and at least 6 weeks of age, were purchased from ENVIGO (Frederick, MD). β-arrestin 2 wild type (WT) and knockout (KO) male mice (25-30 g) were obtained from Dr. Lefkowitz (Duke University, Durham, NC). All animals were housed up to five per cage in animal care quarters and maintained at 22±2°C on a 12-hour light-dark cycle. Access to food and water was available ad libitum. Protocols and procedures were approved by the Institutional Animal Care and Use Committee (IACUC) at Virginia Commonwealth University Medical Center and comply with the recommendations of the International Association for the Study of Pain (IASP).

Acute Tolerance Model. The model for inducing acute tolerance to oxycodone was adapted from a previously published protocol for inducing acute morphine tolerance (Fairbanks and Wilcox, 1999). Lorcaserin or saline pretreatments (2 mg/kg, s.c.) were administered 30 minutes before opioid treatment. Mice were made acutely tolerance to oxycodone by a single gavage of oxycodone (100 mg/kg, p.o.) or saline. Twenty-four hours after opioid exposure, mice were tested for tail withdrawal latencies to ensure that they had returned to baseline values. All subjects were then administered a challenge dose of oxycodone (12 mg/kg, p.o.) and tail withdrawal latencies were assessed 20 minutes later.

Four-Day Tolerance Model. Tolerance to oral oxycodone was developed by administering a twice-daily gavage of oxycodone [64 mg/kg, by mouth (p.o.)] in the morning and the evening, with at least 8 to 10 hours between administrations. Lorcaserin (2 mg/kg, s.c.) was administered 30 minutes before oxycodone gavage, twice daily for four days. This model is reported in Jacob et al. (2017) and adapted from a previously published protocol developed for inducing morphine tolerance (Bernstein and Welch, 1998). Animals were weighed daily and drug volumes were adjusted accordingly. To ensure overall health and hydration, animals received additional subcutaneous injections of saline for the duration of the treatment. The final maintenance dose was on the evening of day 4 and all animals received challenge treatments on day 5 and did not receive lorcaserin treatment. Drug volume was calculated for 0.1mL/10g body weight. All mice had access to *ad libitum* food and water access throughout the treatment and were grouped-housed in home cages.

Warm Water Tail-Withdrawal Test. The warm water tail withdrawal test (52° C) used to assess antinociception in mice was developed by D'Amour and Smith (1941) but modified by Dewey et al (1970). Before drug administration, the baseline (control) latency for each mouse was determined and only mice with a control reaction time from 2 – 4 seconds were used. The test latency after drug treatment was assessed 20 minutes after drug administration, with a maximum cut-off value of 10 seconds to prevent tissue damage to the tail. Antinociception was quantified according to the method of Harris & Pierson (1964) as the percentage of maximum possible effect (%MPE) which was calculated ad: %MPE = [(test control – control)/(10 – control)]x100.

Experimental Design for cumulative dosing protocol. Oxycodone was administered using a cumulative dosing technique. After treatment with the 4-day dosing protocol described above on day 5, the first dose of opioid was administered via oral gavage or subcutaneous injection and animals were tested 20 minutes later. On testing day, animals only received oral oxycodone challenges. After each round of testing, animals received an additional cumulative dose of opioid and tested again 20 minutes later. Testing and dosing continued until the animal reached the maximum cut-off time of 10 seconds.

Gastrointestinal Motility Study. Measurement of total gastrointestinal transit was assessed using the carmine red dye assay. Mice were treated using the four-day tolerance paradigm described above to induce constipation and on the fifth day, GI transit time was assessed. Mice that received the 4-day treatment of chronic oxycodone were observed to enter spontaneous withdrawal on the 5th test day. The carmine red dye assay occurs over a period of several hours and to reduce withdrawal symptoms, mice that received the chronic oxycodone treatment received a low dose of oral oxycodone (10 mg/kg, p.o.) Carmine was suspended in water containing 0.5% methylcellulose and administered intragastrically via gavage at a dose of 0.1mL/10g bodyweight. Immediately after administration of carmine dye, mice were left in separate empty cages until expulsion of a red fecal boli.

Isolation and Culture of Primary Cells from Adult Mouse Dorsal Root Ganglia. DRGs from the adult mouse were prepared as described (Gracious R Ross *et al.*, 2012). Mice were sacrificed via CO_2 inhalation followed by cervical dislocation. L5-S1 DRGs were immediately harvested under a dissecting microscope and placed in a dish containing HBSS. Papain [15 U/ml] was then added to the dish and incubated for 18 min at 37°C. Subsequently, ganglia were transferred to a separate dish containing HBSS and 1.5 mg/ml collagenase from *Clostridium histolyticum* and incubated for 60 min at 37°C. After incubation, ganglia were transferred to DMEM in a sterile 15mL conical flask, dissociated by triturating and centrifuged for 5 min at 1000 rpm. The supernatant was discarded and the pellet was re-suspended in neurobasal A media containing 1% fetal bovine serum, 1x B-27 supplement, 10 ng/mL GDNF, 2mM L-glutamine and 100 U/ml penicillin/streptomycin/amphotericin B (complete neuron media). Isolated cells were plated on laminin and poly-D-lysine-coated glass cover slips and maintained at 37°C in a humidified 5% CO_2/air incubator. Where indicated, isolated neurons were exposed to 10 µM oxycodone and/or 200 nM lorcaserin in complete neuron media for 18-24 hours prior to whole-cell patch-clamp experiments.

Electrophysiology. Methods were used as previously described in Jacob *et al.* (2018). Patch micropipettes were pulled from 1.5/0.84 OD/ID (mm) borosilicate glass capillaries (World Precision Insturuments, Sarasota, FL) on a Flaming/Brown Micropipette puller P97 (Sutter Instruments, Novato, CA) and fire polished. Initial pipette resistances were 2–4 MΩ when filled with filtered internal solution containing (in mM): 100 L-aspartic acid (K salt), 30 KCl, 4.5 Na_2ATP, 1 $MgCl_2$, 10 HEPES, and 0.1 EGTA (pH adjusted to 7.2). Current-clamp experiments were conducted by transporting coverslips containing adhered DRG neurons to a microscope stage plate and superfusing with HEPES-buffered external solution containing (in mM): 135 NaCl, 5.4 KCl, 0.33 NaH_2PO_4, 5 HEPES, 1 $MgCl_2$, 2 $CaCl_2$, and 5 glucose (pH adjusted to 7.4 with NaOH). Because small-diameter neurons correspond to nociceptive Aδ fiber and C-type neurons, only small neurons (<30 pF capacitance) were used (pF = 16.06 ± 0.64, n = 64) (Abraira and Ginty, 2013; Barabas *et al.*, 2014). Whole cell current-clamp recordings were made at room temperature using an Axopatch 200B amplifier (Molecular Devices, Sunnyvale, CA), with a set protocol consisting of 0.01 nA steps beginning at -0.03 nA to assess both active and passive cell properties.

Values reported did not reflect corrected junction potentials (~ -12 mV). Pulse generation and data acquisition were achieved with Clampex and Clampfit 10.2 software (Molecuar Devices, Sunnyvale, CA). Action potential (AP) derivatives were determined using the differential function in Clampfit software, by taking the derivative of the voltage with respect to time (dV/dT). Threshold potentials were defined as the voltage at which dV/dt deviated significantly from zero during the course of an action potential uprise. Assessment of acute oxycodone effects began after a 2-3 min equilibration period, where an external solution containing 3 μM oxycodone solution was then superfused over neurons. Threshold potentials were determined from the first-derivatives of current clamp recordings taken at 1 min intervals for 10 min following oxycodone exposure. The difference between threshold potential values at 0 and 10 minutes was calculated for each cell. Tolerance to oxycodone was assessed in an identical manner in cells that had been incubated overnight in media containing 10 μM oxycodone. The effect of lorcaserin on oxycodone tolerance was assessed by incubating cells overnight in 10 μM oxycodone and 200 nM lorcaserin. The following day, neurons were then superfused with external solution containing 3 μM oxycodone. In all experiments, "N" represents the total number of mice and "n" represents the total number of cells within each group from which recordings were obtained.

Binding Assay. Mice were treated using the 4-day chronic oxycodone paradigm and on the fifth day, spinal cord tissues were dissected and then flash frozen in liquid nitrogen. For obtaining membrane homogenates, tissues samples were homogenized in a HEPES buffer (in mM 20 HEPES, 10 MgCl2, 2 EGTA, and 100 NaCl, pH 7.7) containing 0.25 M sucrose using a telfon-glass dounce homogenizer. The homogenates were centrifuged at 1000xg for 10 minutes at 4C and then the pellet was discarded. The supernatant was centrifuged again at 40,000xg for 15 minutes and the remaining pellet was washed twice with homogenization buffer and then subsequenty respun after each washing at 40,000xg for 20 minutes. The final pellet was kept at -80C until use. On test day, the pellet was suspended in assay buffer and protein concentrations were assessed using the Bradford method and 7 – 10 ug of protein were used per data point. Assay conditions were developed based on Leitchi et al. (2007). Spinal cord membranes were run in triplicate for 45 minutes in assay buffer at 30C with 10uM GDP, 0.1nM [35S]GTPyS, and in the presence or absence of opioid agonist (10nm – 1mM, DAMGO). Basal [35S] GTPyS binding was determined in the absence of opioid agonist. Nonspecific binding was measured with 20uM unlabeled GTPyS and specific binding was determined by substracting nonspecific binding from total binding. The binding reaction was termined using rapid vacuum filtration through GF/C glass fiber filters using a harvester (Brandel, Gaithersburg, MD) and washed 3 times with ice cold assay buffer. Filters were allowed to dry for one hour and then bound radioactivity was determined using the Liquid Betaplate Scintillation counter (Wallace SC/9200/21, PerkinElmer 1205-440).

Data Analysis. All data are reported as mean values ± S.E.M. from experiments that were performed in at least duplicate. For the binding studies, nonspecific binding was substrated from total [^{35}S]GTPγS binding and net agonist-stimulated [^{35}S]GTPγS binding is defined as agonist-stimulated binding minus basal binding. Non-linear aggression analyses of concentration-effect curves were performed using GraphPad Prism software (GraphPad Software, Inc., La Jolla, CA). Statistical significance was determined using two-way analyses of variance (2-way ANOVA) with drug treatment and the concentration of agonist used as the independent variables. If significance was detected, the data was subject to a Tukey's post-hoc analysis.

For the electrophysiology data, statistical differences were calculated using GraphPad Prism 5.0 (GraphPad Software, Inc., La Jolla, CA). For All analyses were conducted on the small "n" value, representing total cell numbers (except for Figure 5, where the "N" representing the number of mice was analyzed). Within-subject comparisons were analyzed via Student's paired t-test. For group comparisons, results were analyzed by two-way ANOVA with Bonferroni post-hoc test, and an alpha level set to 0.05. The results are expressed as mean value ± SEM, except where individual data points are shown.

V. Results

Lorcaserin blocked the development of acute antinociceptive tolerance.

Acute Tolerance in vivo

The data presented in Figure 3.1 show the effect of lorcaserin (2 mg/kg, s.c.) pretreatment on the development of acute antinociceptive tolerance to the warm-water tail withdrawal test. On challenge day, all animals displayed normal baseline tail-withdrawal behavior (2 – 4 seconds to remove their tails from the water). All animals were administered the same challenge dose of oxycodone (12 mg/kg, p.o.). Control mice that received only saline produced a maximal antinociceptive response of 50.69 %MPE. Lorcaserin treatment on its own did not significantly alter the acute effect of the oxycodone challenge compared to saline controls. Pretreatment with the large dose of oxycodone (100 mg/kg, PO) on day 1 produced a significant reduction in the maximal possible effect of the challenge dose from 50.69% to 23.91 %MPE in the tolerant animals ($P < 0.05$, one-way ANOVA). The combination treatment of lorcaserin and the large dose of oxycodone on day 1 significantly altered the effect of the challenge dose of oxycodone (64.88 %MPE) compared to tolerant mice ($P < 0.05$, one-way ANOVA) and this level of antinociception was not significantly different from saline controls.

Overnight exposure to oxycodone *in vitro* led to tolerance. Previously, our lab has demonstrated that overnight incubation with 10 μM oxycodone in vitro leads to a tolerant phenotype (Jacob *et al.*, 2018). Neurons were incubated overnight for a minimum of 18 hours with 10 μM oxycodone before being moved to the microscope stage plate which contained an external solution with no drug treatment. Cells were challenged in the bath with a 10-minute treatment of 3 μM oxycodone. Control neurons produced a significant shift in threshold potential of +4.10 ± 1.30 mV relative to their baseline threshold values (*P*-value < 0.0002). Cells incubated overnight with 10 μM oxycodone did not produce a change in threshold potential (-25.36 ± 4.207 mV v.s. -23.183 ± 3.719 mV; *P* > 0.05) after 3 μM oxycodone challenge, indicating that tolerance had developed (Figure 3.4).

Overnight co-incubation with lorcaserin and oxycodone results in an attenuation of acute tolerance in vitro. To further test the hypothesis that lorcaserin will attenuate the development of acute tolerance in vitro, neurons were incubated overnight with 10 μM oxycodone and 200 nM lorcaserin (Figure 3.4). Following overnight exposure, neurons were assessed for baseline threshold potentials and then perfused for 10 minutes with a 3 μM oxycodone challenged. Upon challenge, the neurons displayed a significant shift in the threshold potential and demonstrated a +3.75 ± 1.42 mV increase, from -13.41 ± 1.52 to -9.16 ± 1.33 mV (*P* < 0.05), and shifted similar to cells observed under acute, non-tolerant oxycodone conditions. Furthermore, overnight treatment with 1 μM SB242084, a selective 5-HT$_{2C}$ receptor antagonist, oxycodone, and lorcaserin returned threshold recordings to that of the tolerant phenotype, with no change in threshold potential (-14.55 ± 2.55 mV v.s. -16.66 ± 1.98 mV) after 3 μM oxycodone challenge.

Lorcaserin partially attenuated the development of multiple-day tolerance.

Multiple-day tolerance in vivo. Tolerance to the antinociceptive effect of oxycodone was induced using our 4-day tolerance model. The ED$_{50}$ values for the treatment groups are listed in Table 3.1. The dose-response curves from which the ED$_{50}$ values were generated for the treatment groups are shown in Figure 3.2. On day 5, mice only received cumulative doses of oxycodone and did not receive an additional pretreatment. All animals display normal baseline values for latency to withdraw their tails from the water (2 – 4 seconds). Saline control mice produced an ED_{50} of 5.39 (4.3 – 6.74). Chronic oxycodone treatment [F(1, 126) = 167.7, $p < 0.0001$] produced a significant 4-fold shift in the ED_{50} value to 19.56 (17.01 – 22.48). The ED_{50} value for the animals that received only the lorcaserin treatment (and no oxycodone) did not significantly differ from saline controls [F (1, 126) = 2.93, P = 0.089), although a trend toward increased potency was observed. Mice that were treated with both lorcaserin and oxycodone displayed a partial attenuation of the development of antinociceptive tolerance [F (1, 119) = 17.76, $p < 0.0001$], as denoted by the significant shift in the ED_{50} value (9.53, 7.84 – 11.59) relative to the tolerant mice and saline controls. Two-way ANOVA indicated significant main effects of drug treatment and dose for all drugs (*p*-value < 0.0001).

Lorcaserin did not alter the constipating effect of chronic oxycodone. The constipating effect of chronic oxycodone was assessed using the 4-day treatment paradigm and on day 5, total gastrointestinal transit time was assessed using the carmine dye assay. The data presented in Figure 3.3 shows the effect of lorcaserin pretreatment on the chronic effect of oxycodone. Saline control mice displayed a mean GI transit time of 77.90 minutes and treatment with chronic oxycodone produced a significant increase in total transit time to 112.6 minutes ($P < 0.001$, one-way ANOVA). Lorcaserin treatment alone did not alter transit time and mean time to expulsion of red bolus was 84.56 minutes. Combination treatment with lorcaserin and oxycodone did not block the constipating effect of chronic oxycodone, as the mean transit time was 113.0 minutes, and these mice were significantly different from saline controls ($P < 0.01$, one-way ANOVA).

Effect of chronic oxycodone and chronic lorcaserin treatment on DAMGO-stimulated [^{35}S]GTPγS binding. Mu opioid receptor-stimulated [^{35}S]GTPγS binding was examined in control and chronic drug-treated mice after 4 day treatment *in vivo* (Figure 3.5). Binding was assessed to determine whether chronic treatment with lorcaserin produced a change in MOR-mediated G-protein activation following chronic oxycodone exposure. The concentration-effect curves were generated using the MOR-selective full agonist DAMGO in spinal cord membrane homogenates prepared from mice injected with saline, lorcaserin alone, chronic oxycodone alone, or chronic lorcaserin and oxycodone. Treatment with chronic oxycodone [F (1, 126) = 303.3, P < 0.0001] showed concentration-dependent reduction in DAMGO-stimulated [^{35}S]GTPγS binding and a ~38% decrease in the E_{Max} value of relative to saline control mice but with no significant difference in the EC_{50} values (*P* < 0.001, Two-way ANOVA with multiple comparisons) (Table 3.2). There was no difference in E_{Max} or EC_{50} values between saline treated or lorcaserin only treated mice [F (1, 95) = 2.475, P = 0.119]. Treatment with combination chronic lorcaserin and oxycodone showed a similar significant concentration-dependent reduction in DAMGO-stimulated [^{35}S]GTPγS binding but only at 1, 10 and 100 μM concentrations (*P* < 0.0001, two-way ANOVA with multiple comparisons) and an approximate ~35% reduction in E_{Max} and no change in EC_{50} value relative to vehicle controls [F (1, 126) = 187.2, P < 0.0001]. Basal [^{35}S]GTPγS binding significantly differed between saline controls and chronic oxycodone alone (*P* < 0.0001) and significantly differed between chronic oxycodone and combination chronic lorcaserin and oxycodone (*P* < 0.05).

VI. Discussion

To determine the effect of lorcaserin on the development of opioid tolerance, a model of both acute (or short-term) tolerance and long-term (chronic) tolerance were used. It should be noted that there are distinct differences in the type of tolerance that develops after these treatment paradigms and they differ in their mechanisms of induction (Rosenfeld *et al.*, 1977; Fairbanks and Wilcox, 1997). The short-term model is suggested to be mediated through acute desensitization that occurs in a period of minutes to hours and is considered to be the initiation stage of tolerance (Cox *et al.*, 1968; Sibley *et al.*, 1984, 1987; Hausdorff *et al.*, 1989; Kovoor *et al.*, 1998; Lefkowitz, 1998; Whistler and von Zastrow, 1998; Laura M. Bohn *et al.*, 2000; Borgland *et al.*, 2003; Bohn *et al.*, 2004). Chronic, multiple-day, models of tolerance are thought to be mediated through counter-adaptive changes in intracellular signaling and receptor downregulation that occurs over the period of a few days to weeks (Tempel and Zukin, 1987; Tempel, 1991; Tao *et al.*, 1993; Z Wang *et al.*, 1994; Wang *et al.*, 2004; Sim-Selley, 2005; Shoblock and Maidment, 2006; Sim-Selley *et al.*, 2009).

It should be noted that the dose of lorcaserin (2 mg/kg, s.c.) that was chosen for these studies is an active dose that produced a significant shift in the ED_{50} value of acute oxycodone (see Chapter 2 of this dissertation). These evaluations of lorcaserin's effects on opioid tolerance utilized the 2 mg/kg dose but future studies should evaluate additional doses of lorcaserin.

Acute Tolerance & Lorcaserin

Treatment with a single, large dose of oral oxycodone produced significant acute tolerance, in agreement with studies previously conducted with morphine (Cox *et al.*, 1968; Huidobro-Toro and Way, 1978; Wigdor and Wilcox, 1987; Laura M. Bohn *et al.*, 2000). Pretreatment with lorcaserin (2 mg/kg, s.c.) did not have a significant effect on its own although there was a trend towards increased efficacy of the oxycodone challenge on the following day. More importantly, the lorcaserin pretreatment significantly blocked the development of the acute tolerance.

Furthermore, this model can be extended to an *ex vivo* evaluation of DRG neurons. Several studies have validated the use of DRGs as a model of opioid tolerance (Gracious R Ross *et al.*, 2012; Kang *et al.*, 2017; Jacob *et al.*, 2018). DRGs are a useful model for evaluating neuronal tolerance on a single cell level and DRGs are nociceptive afferents that transmit incoming stimuli to the central nervous system. This is an ideal model for evaluating the effect of lorcaserin on opioid tolerance. The studies by Jacob et al. (2016) have demonstrated that overnight exposure to oxycodone (10 μM) produced reproducible tolerance as evidenced by changes in threshold potential.

DRG neurons incubated overnight with 10 μM oxycodone and then challenged with 3 μM oxycodone following the overnight exposure did not exhibit a change in threshold potential, indicating that tolerance had developed. This tolerance was blocked by a co-incubation with 200 nM lorcaserin (a dose chosen based on its relative EC_{50} between the 5-HT_{2C} and 5-HT_{2A} receptors) and these cells demonstrated shifts in threshold potential similar to that of naïve cells (Thomsen *et al.*, 2008). Furthermore, this effect of lorcaserin was blocked by SB242084, a selective 5-HT_{2C} receptor antagonist, and suggests that the effect of lorcaserin on opioid tolerance is indeed mediated through the 5-HT_{2C} receptor.

These data further support the findings *in vivo* where lorcaserin completely blocked acute tolerance in the whole animal and blocked the development tolerance on the single cell level. The electrophysiology data suggest that the 5-HT_{2C} receptor is, in fact, expressed outside of the central nervous system, a finding that does not agree with the current dogma in the literature (Chen *et al.*, 1998; Clemett, *et al.*, 2000; López-Giménez *et al.*, 2001; Nicholson *et al.*, 2003).

The effect of lorcaserin on multiple-day (long-term) tolerance.

In the long-term model of oxycodone tolerance, mice treated with both oxycodone and lorcaserin develop tolerance to the antinociceptive effects of oxycodone but to a lesser extent compared to their chronic oxycodone alone controls. Chronic treatment with oxycodone produced an approximate 4-fold shift in the ED_{50} relative to the mice that only received only the oxycodone challenges and chronic saline treatment. Chronic lorcaserin treatment alone did not significantly alter the acute effect of oxycodone and did not produce a significant shift in the relative ED_{50}. The ED_{50} of chronic lorcaserin and oxycodone animals was significantly different from both saline-treated animals and chronic oxycodone-treated animals. The data from both the acute tolerance and long-term tolerance models suggest that the role of the 5-HT_{2C} receptor in the mechanisms of tolerance is different. This is in support of the observation that the mechanisms that underlie acute and long-term tolerance are distinct from one another.

In addition, the effect of lorcaserin in the long-term model of antinociceptive tolerance appears to be different than its effects on chronic opioid-induced constipation. A common side effect of chronic opioid use is constipation (Shook *et al.*, 1987; Ling *et al.*, 1989; Ross *et al.*, 2008; Tuteja *et al.*, 2010). The long-term tolerance model produced significant constipation relative to mice that were only treated with chronic saline. Chronic lorcaserin alone did not have any significant gastrointestinal effects and in combination with chronic oxycodone, also did not alter the constipating effects. This is not entirely surprising, however, as the 5-HT_{2C} receptor is not known to be expressed in the gastrointestinal system nor it is known to contribute to any gastrointestinal functions (Fiorica-Howells *et al.*, 2000). In clinical trials of lorcaserin, approximately ~6% of patients experienced diarrhea or constipation as an adverse reaction and presently, the direct effects of 5-HT$_{2C}$ receptor activation on gastrointestinal function remains to be elucidated (FDA, 2016).

As the antinociceptive effects of oxycodone are predominantly mediated through the MOR, these studies examined the ability of lorcaserin to alter the desensitization state of the MOR (Matthes *et al.*, 1996; Sora *et al.*, 1997; Kitanaka *et al.*, 1998; Loh *et al.*, 1998; Weibel *et al.*, 2013). Chronic administration of an opiate, such as morphine, has been shown to induce uncoupling of G-proteins from opioid receptors and it has been proposed that this phenomenon may, in part, underlie tolerance (Sim *et al.*, 1996; Smith *et al.*, 2007; Priyanka A Madia *et al.*, 2012). The spinal cord is thought to be a major component in the elicitation of antinociception by opioids and exhibits MOR desensitization and downregulation following chronic morphine treatment (Sim-Selley *et al.*, 2009). Using spinal cord membrane homogenates from animals chronically treated with oxycodone and/or lorcaserin, DAMGO-stimulated [^{35}S]GTPγS binding was assessed. Treatment with chronic oxycodone alone, relative to saline controls, produced a significant reduction in basal receptor activity (binding in the absence of an agonist) and a ~38% decrease in E_{Max}. Chronic treatment with lorcaserin did not alter basal binding or maximal amount of [^{35}S] GTPγS bound relative to controls. Combination treatment with both chronic lorcaserin and chronic oxycodone resulted in a significant increase in basal activity relative to animals that received chronic oxycodone alone and a significant effect at the lowest dose of DAMGO tested (10 nM). This study suggests that lorcaserin is not altering desensitization of the MOR and is working to alter tolerance through a mechanism that is likely independent of MOR function. The overall reduction in basal receptor activity following chronic morphine begs the question of changes in receptor constitutive activity or receptor downregulation that requires further evaluation.

Conclusions

The effect of lorcaserin on chronic oxycodone-induced antinociceptive tolerance is a dosing-frequency and time-dependent phenomenon that is evidenced by the differential effects of lorcaserin in the acute and chronic models of opioid tolerance. Although the frequency of drug administration varies greatly between the two paradigms, it is possible that lorcaserin's effects are dependent both upon the frequency of drug exposure and the time in which lorcaserin is administered. Lorcaserin is capable of fully attenuating the development of acute tolerance but only partially reversed antinociceptive tolerance in the long-term model. This suggests that the role of the 5-HT$_{2C}$ receptor in these stages of tolerance is dependent upon different mechanisms. The expression of the 5-HT$_{2C}$ receptor throughout the CNS is overall very low but does display distinct localization in the dorsal horns of the spinal cord. Colocalization of the 5-HT$_{2C}$ receptor and MOR has not been investigated so it is difficult to speculate whether proximity on the same neuron is a contributing factor. Clearly, lorcaserin is altering the early stages of tolerance/desensitization but the mechanism through which is unclear. Possible roles of the 5-HT$_{2C}$ receptor in altering opioid tolerance is through changes in receptor phosphorylation and recruitment of kinases, such as GRK or PKC. Although the mechanism is not clear, the potential of lorcaserin and oxycodone as a combination treatment, as it is favorable altering the development of tolerance.

These data demonstrate that lorcaserin has differential effects on different models of tolerance and this provides some insight into different roles of the MOR and the 5-HT2c receptor. Previous studies have only evaluated the effect of lorcaserin on self-administration and naloxone-precipitated withdrawal in opioid dependent rodents. In addition to having characterized the acute effects on opioid antinociception, this is the first series of studies that has shown that lorcaserin also alters the antinociceptive tolerance that develops to opioids.

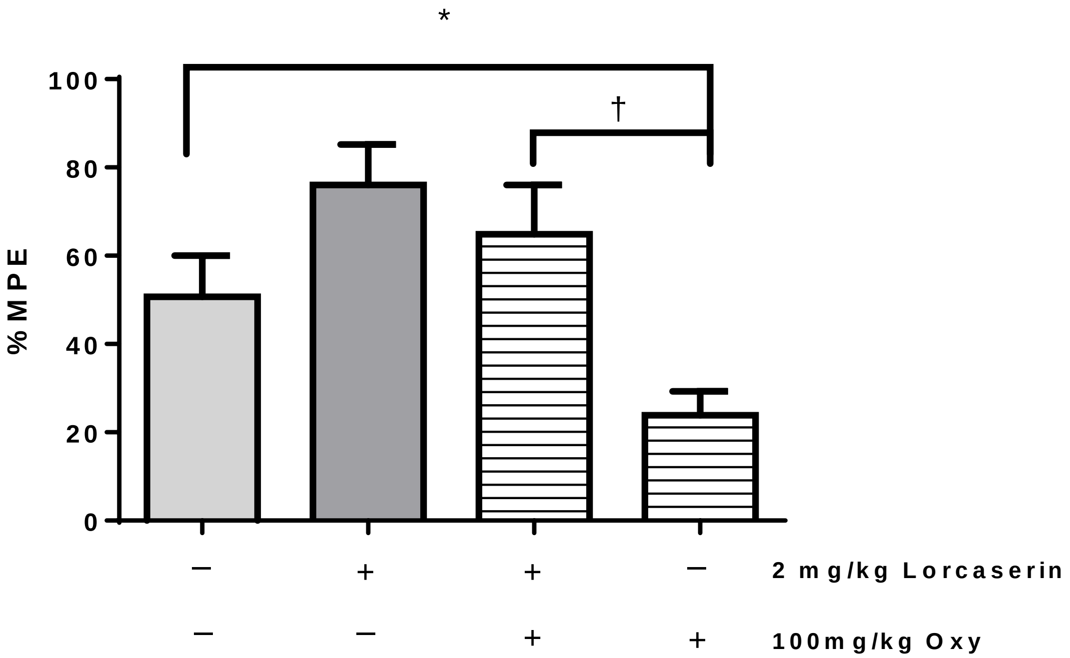


Figure 3.1: Lorcaserin pretreatment attenuated the development of acute antinociceptive tolerance. The day after tolerance induction, all animals received a challenge dose of oxycodone (12 mg/kg, p.o.) and were tested for antinociceptive responses. Acute tolerance was induced and there was a significant reduction in %MPE to the oxycodone challenge and treatment with lorcaserin (2 mg/kg, s.c.) blocked this effect. At least 8-10 mice were used per group and the experiment was repeated twice. † $P < 0.05$ using one-way ANOVA with multiple comparisons, 2 mg/kg lorcaserin + 100 mg/kg oxycodone v.s. saline + 100 mg/kg oxycodone. *$P < 0.05$ using one-way ANOVA with multiple comparisons.

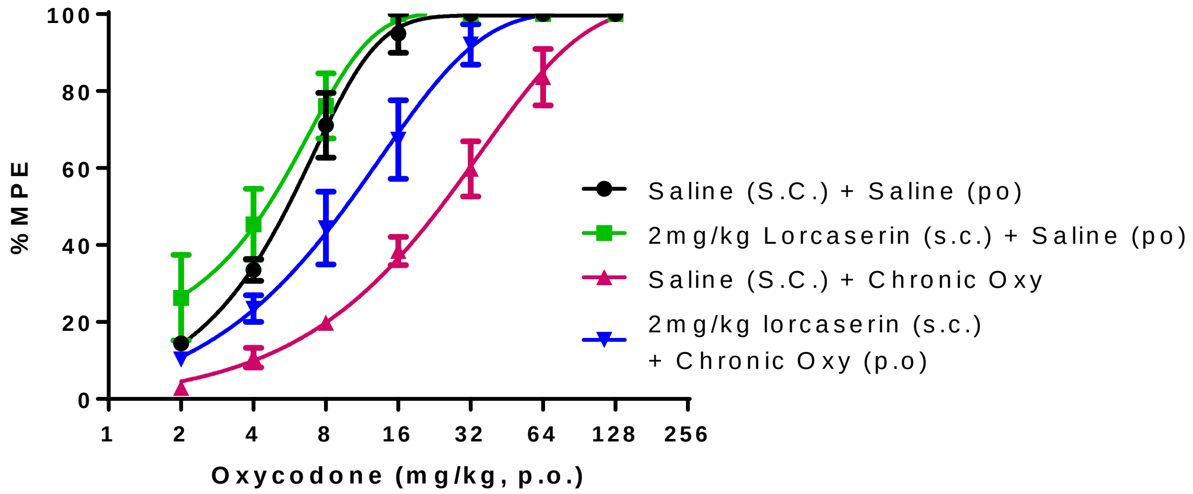


Figure 3.2: Lorcaserin partially attenuated the development of multiple-day antinociceptive tolerance. Mice were administered 4-day treatment of saline, lorcaserin (2 mg/kg, s.c.), and/or oxycodone (64 mg/kg, p.o.). On the fifth day, animals were challenged using a cumulative dosing procedure of oral oxycodone. All points represent the mean ± S.E.M. and ten animals per group were tested across two separate days.

Group	ED50	95% CL
Saline + Saline	5.39	4.3 – 6.74
Lorcaserin + Saline	3.99	2.37 – 4.86
Saline + Chr. Oxy	19.56*	17.01 – 22.48
Lorcaserin + Chr. Oxy	9.53*	7.84 – 11.59

Table 3.1: ED50 values (mg/kg) and 95% confidence limits for long term antinociceptive tolerance experiment shown in Figure 3.2. Antinociceptive tolerance was assessed using cumulative dosing in the warm-water tail withdrawal test. Significant shifts from saline + saline control values are denoted by “*”.

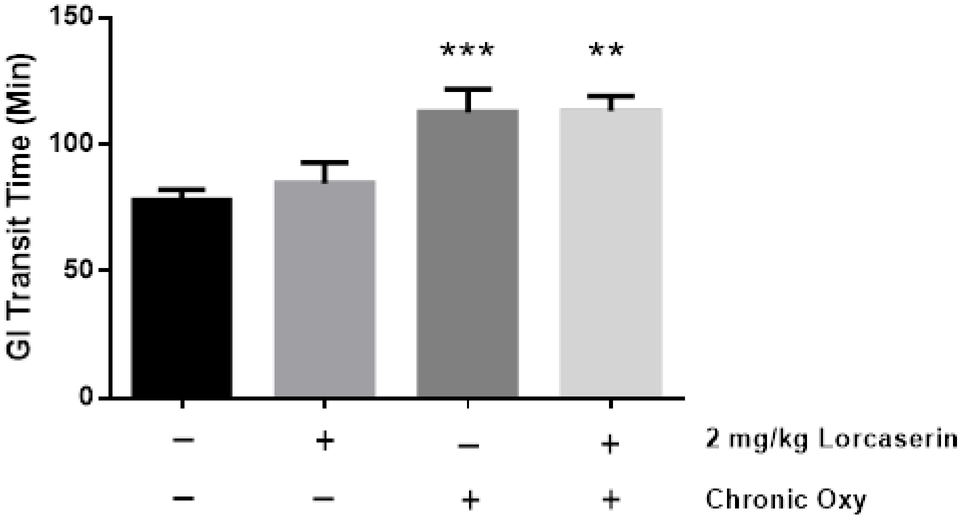


Figure 3.3: Lorcaserin did not alter the constipating effect of chronic oxycodone using the 4-day tolerance paradigm in the carmine red dye assay. Animals were assessed for gastrointestinal transit time on the fifth day. At least 9-10 mice were used per treatment group. ** $P < 0.01$ and *** $P < 0.001$ from saline + saline control using one-way ANOVA with Dunnett's post-hoc.

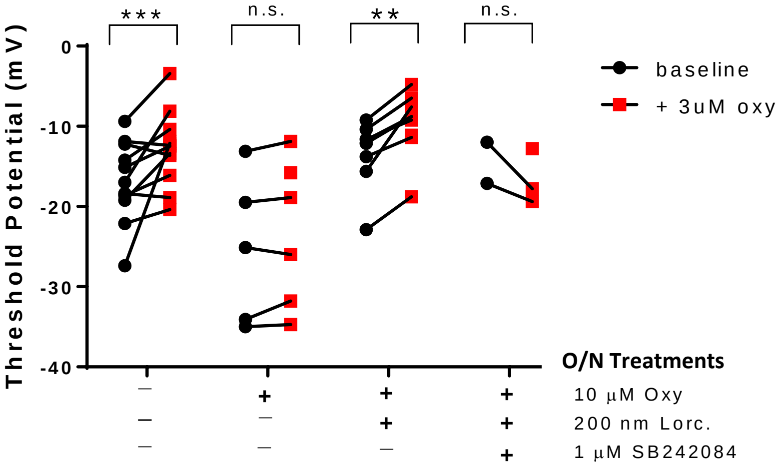


Figure 3.4: Threshold potentials in response to 3 µM oxycodone challenge after overnight incubation with 10 µM oxycodone, 200 nM lorcaserin, and 1 µM SB242084. Ten-minute treatment in the bath with 3 µM oxycodone produced a significant shift in the threshold potential of untreated DRG neurons (****P*-value < 0.001), and in neurons incubated overnight with both 10 µM oxycodone and 200 nM lorcaserin (***P*-value < 0.05). Neurons incubated overnight with 10 µM oxycodone alone or 10 µM oxycodone and 1 µM SB242084 did not significantly respond to treatment with 3 µM oxycodone (n.s., non-significant). Data represents individual changes in cell threshold potentials before (●) drug treatment and 10 minutes after (red ■) 3 µM oxycodone treatment in the bath. Statistical significance was assessed using a two-way repeated measures analysis of variances with a Bonferroni's post-hoc test and deemed significant if *P* < 0.05.

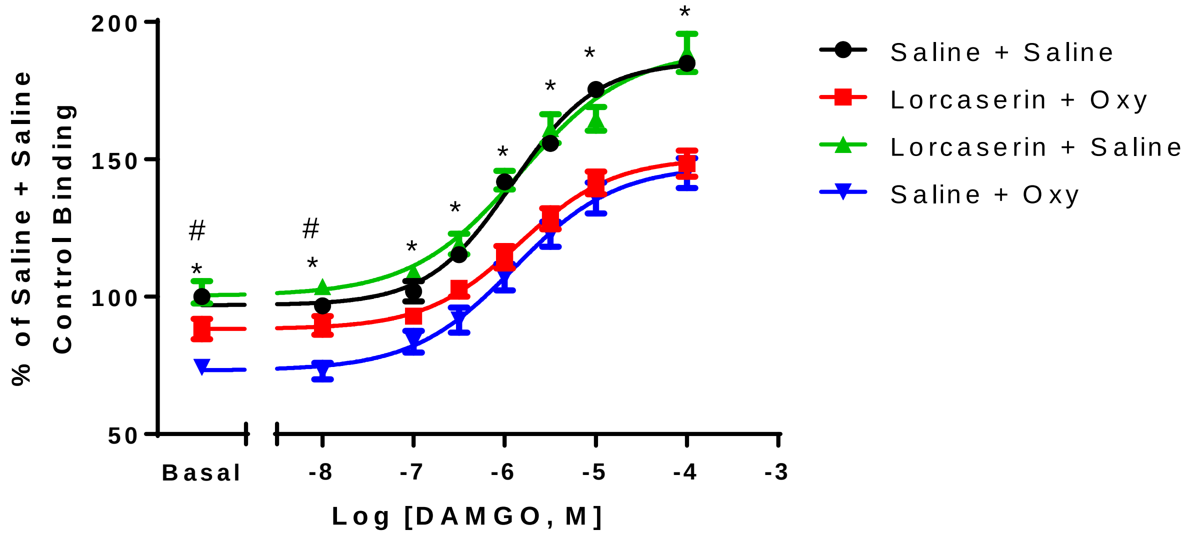


Figure 3.5: Effect of chronic oxycodone and chronic lorcaserin treatment on DAMGO-stimulated [^{35}S]GTPγS binding. After 4-day treatment of chronic oxycodone (64 mg/kg, p.o., b.i.d.) and/or chronic lorcaserin (2 mg/kg, s.c., b.i.d.) spinal cords were dissected and were incubated with 0.1 nM [^{35}S]GTPγS, 10 µM GDP and the indicated concentrations of DAMGO. Significant MOR desensitization was observed between animals that received chronic oxycodone versus vehicle controls (**P* <0.0001, Two-way ANOVA with multiple comparisons). Animals that received chronic lorcaserin and chronic oxycodone demonstrated significantly different basal activity and at the lowest dose of DAMGO activity (#*P* <0.05, Two-way ANOVA with multiple comparisons). Data were analyzed by nonlinear regression (GraphPad Prism) and presented as the % of control mice biding ± S.E.M. Curves are performed in triplicate in which control (saline + saline) mice and chronic oxycodone alone were assayed simultaneously.

Treatment Group	**E_{Max} (%Control)**	**$LogEC_{50}$ ± SE**
Chronic Saline + Chronic Saline	185.6 ± 2.5	-5.91 ± 0.04
Chronic Lorcaserin + Chronic Saline	189.7 ± 6.4	-5.83 ± 0.13
Chronic Saline + Chronic Oxycodone	147.7 ± 5.8	-5.84 ± 0.12
Chronic Saline + Chronic Lorcaserin	150.5 ± 4.5	-5.89 ± 0.14

Table 3.2: Effect of chronic oxycodone and/or lorcaserin combination treatment on E_{Max} and $logEC_{50}$values on mu opioid-receptor-stimulated [^{35}S]GTPγS binding. Spinal cord membrane homogenates from treated mice were incubated with 0.1 nM [^{35}S]GTPγS, 10 μM GDP and varying concentrations of DAMGO as described in the Methods. Data are demonstrated as E_{Max} and $logEC_{50}$ values ± SE were derived from the concentration-effect curves shown in Figure 5.

Chapter 4

General Discussion

Opioids are a class of compound that have demonstrated continued use in the clinic for the treatment of pain in spite of their risks. While efficacious, continued use of opioids is associated with the development of analgesic tolerance, dependence, and in some unfortunate cases, increased risks of mortality due to its respiratory depressive effects (Gomes *et al.*, 2011). The over-prescribing of opioids and their associated side-effects has led to the development of the present opioid epidemic that claims over 100 lives every day (CDC *et al.*, 2016). Although the recommendations for the prescription of opioids by practitioners has changed in light of this epidemic, the need for alternative therapies is ever present (Dowell *et al.*, 2016b; F Collins *et al.*, 2017). Opioid-sparing adjunctive therapies have been explored and several alternatives have been identified: non-steroidal anti-inflammatories, gabapentoids, acetaminophen, and antidepressants (Saarto and Wiffen, 2007; Buvanendran and Kroin, 2009; Derry *et al.*, 2009, 2013; Gaskell *et al.*, 2009; Straube *et al.*, 2010; Gilron, 2016; Sullivan *et al.*, 2016). Each combination and therapy vary in their efficacies, potential toxicity to patients, and are not equally efficacious in treating pain.

The notion of "opioid-sparing" was approached with two concepts in mind. First, in the event of first exposure with an opioid, opt for a lower dose because lower doses in cases of initial exposures are associated with a reduced likelihood of long-term abuse (Shah *et al.*, 2017) Second, in the case of long-term use, administration of a lower dose of opioid will produce less toxic side effects, such as dependence, tolerance, and constipation, and overall be safer for the patient. Therefore, with this approach in mind, the overall goal of these studies was to identify a novel opioid-sparing adjunct that altered both the acute and chronic effects of opioids, with a primary focus on the prescription opioid oxycodone. The studies described herein advance our knowledge of the role of lorcaserin, and the 5-HT_{2C} receptor through which it exerts its effects, in the acute antinociceptive effects of opioids and its interactions in the development of opioid tolerance at the behavioral and cellular level.

There are three major conclusions that can be derived from these studies and which will be subsequently elaborated on below. First, the effect of lorcaserin in a preclinical model of acute thermal pain is site-specific. Second, lorcaserin enhanced the acute antinociceptive effects of several opioids and increased the time course of oxycodone's antinociceptive activity through activation of the 5-HT_{2C} receptor. Finally, lorcaserin blocked the development of antinociceptive tolerance to oxycodone but with a dosing-frequency-dependent effect. Collectively, these data suggest that lorcaserin may be a novel alternative therapeutic adjunct in addition to those that are currently available.

These studies were initiated due to the observation that lorcaserin attenuated oxycodone self-administration and decreased cue reactivity associated with abstinence and relapse in a rodent model of opioid addiction (Neelakantan *et al.*, 2017). Additional studies in rodents that evaluated the therapeutic potential of lorcaserin to suppress remifentanil self-administration found that lorcaserin non-selectively attenuated both food and drug self-administration (Panlilio *et al.*, 2017). Conflicting data evaluating lorcaserin in models of opioid addiction may limit lorcaserin's translation into the clinic but the purpose of this discussion is not to evaluate the therapeutic potential of lorcaserin as a pharmacotherapeutic for addiction. Based on the idea that lorcaserin can alter one aspect of oxycodone's pharmacology, the overall goal of these studies was to evaluate the effect of lorcaserin on the acute and chronic antinociceptive effects of oxycodone and specifically from the opioid-sparing perspective.

Over the past several decades, many therapeutics have emerged as possible opioid-sparing adjuncts, with the most notable being cannabinoid compounds, and acetaminophen and NSAIDs (Kolesnikov *et al.*, 2003; Smith *et al.*, 2007; Huang *et al.*, 2008; Derry *et al.*, 2009, 2013; Gaskell *et al.*, 2009; Nielsen *et al.*, 2017). Their preclinical characterization is remarkably similar in that first the compounds themselves were evaluated via several routes of administration (e.g., subcutaneous, intrathecal, intravenous, intracerebroventricular) within their given preclinical model of pain (Lichtman and Martin, 1991; Welch and Stevens, 1992; Raffa *et al.*, 2000). Following this characterization and approximation of possible locus of action, further studies were conducted in combination with an opioid. The initial goal of these studies was to characterize the effect of lorcaserin by itself in a similar manner to that of which has been previously published.

The antinociceptive activity of intrathecally administered 5-HT$_{2C}$ receptor agonists has been characterized for some time, though limited by the lack of selectivity among previously available ligands (Obata *et al.*, 2003, 2004, 2007; Nakai *et al.*, 2010). Lorcaserin was developed as a selective 5-HT$_{2C}$ receptor agonist and had an approximate ~12-fold greater selectivity for 5-HT$_{2C}$ over 5-HT$_{2A}$ (Thomsen *et al.*, 2008). Lorcaserin, in particular, has only been evaluated in a chronic pain model in a study by Ogino *et al.* (2013) where both systemic administration of lorcaserin attenuated mechanical hypersensitivity in a preclinical model of fibromyalgia. A thorough literature search showed that 5-HT$_{2C}$ receptor agonists have only been evaluated in models of neuropathy and chronic pain, and the effect of lorcaserin on acute pain had not yet been investigated.

Using the warm-water tail withdrawal test as a model of acute thermal nociception, the effect of lorcaserin was evaluated via the subcutaneous, intrathecal, and intracerebroventricular routes of administration. In agreement with the previous data on intrathecal administration of 5-

HT_{2C} agonists, lorcaserin produced the expected dose-dependent antinociceptive response. It should also be mentioned that unlike NSAIDs or acetaminophen, its antinociceptive effect was not blocked by naloxone which indicates that its effects are not mediated through the opioid system (Herrero and Headley, 1996; Raffa *et al.*, 2000). Intracerebroventricular administration of lorcaserin was completely inactive and at the highest doses, induced seizures in some animals. This finding was unexpected and suggests that activation of the 5-HT_{2C} receptor in nociception differs between spinal and supraspinal sites.

This sort of phenomenon, however, is not entirely unusual. Acetaminophen is another example of an opioid-sparing agent that displays measurable intrathecal antinociceptive activity but has little to no effect when administered intracerebroventricularly (Raffa *et al.*, 2000). These differences in effects could be due to distinct mechanisms of action that underlie their antinociceptive effects in spinal and supraspinal sites. Acetaminophen, for example, was not equally antagonized by pretreatment with naloxone (Raffa *et al.*, 2000). Intrathecal and subcutaneous administrations of acetaminophen were both antagonized by naloxone but intracerebroventricular administration of acetaminophen was not, suggesting distinct mechanisms of action. Our studies with lorcaserin via intrathecal and intracerebroventricular routes of administration displayed a similar pattern of effect, like acetaminophen, that may be attributed to differences in their spinal and supraspinal mechanisms.

Unlike the study by Ogino (2013) where lorcaserin was active when administered orally in their chronic pain model, subcutaneous lorcaserin was inactive in our model of acute pain. Previously published data and our studies suggest that efficacy of systemically lorcaserin and similar 5-HT_{2C} receptor agonists to treat pain is dependent upon the type of pain (Obata *et al.*, 2004; Nakai *et al.*, 2010; Ogino *et al.*, 2013). Furthermore, chronic pain (where 5-HT_{2C} receptor agonists were effective) displays measurable changes in 5-HT$_{2C}$ receptor expression which may underlie this observed difference in efficacy (Wu *et al.*, 2001; Nicholson *et al.*, 2003; Liu *et al.*, 2005). Administration of intraplantar bee venom or CFA is reported to upregulate 5-HT$_{2C}$ receptor mRNA in dorsal root ganglion neurons and the dorsal horn of the spinal cord (Wu *et al.*, 2001; Nicholson *et al.*, 2003; Liu *et al.*, 2005). Further study investigating the effect of lorcaserin in models of chronic pain are needed as published data suggests that it may be most efficacious in models of chronic pain and not necessarily useful on its own as a treatment for acute pain (Obata *et al.*, 2004; Nakai *et al.*, 2010; Ogino *et al.*, 2013).

After characterizing the effect of lorcaserin in an acute pain model, it was tested in combination with acute doses of opioids. Previous studies have evaluated interactions between 5-HT$_{2C}$ receptor agonists and opioids in models of drug self-administration and addiction, but interactions between 5-HT$_{2C}$ receptor agonists and opioids in the study of pain have not been characterized (Wu *et al.*, 2015; Zhang *et al.*, 2015; Neelakantan *et al.*, 2017; Panlilio *et al.*, 2017). As mentioned previously, the study by Neelakantan *et al.* (2017) and Panlilio (2017) evaluated the effect of lorcaserin in rodent models of opioid self-administration and yielded conflicting results. In addition, two additional studies reported that lorcaserin attenuates behavioral sensitization (a behavior often thought to be associated with the rewarding effects of drugs of abuse) and naloxone-precipitated withdrawal symptom severity in mice dependent upon heroin or morphine (Wu *et al.*, 2015; Zhang *et al.*, 2015). The abuse liability of opioids is a major public health concern, but their wide use as analgesics make it necessary to evaluate opioid and 5-HT$_{2C}$ agonist interactions in a preclinical model of pain and to determine if reduced doses of opioids can be used as a result of this combination.

Analgesics are unlikely to be administered via an intrathecal or intracerebroventricular routes in the clinic (except in special cases), therefore, the combined effects of subcutaneously administered lorcaserin and oxycodone were assessed (Calias *et al.*, 2014; Atkinson, 2017; Cohen-Pfeffer *et al.*, 2017). Antinociceptive responses were assessed using a cumulative dosing method in the warm-water tail withdrawal assay. Several doses of subcutaneous lorcaserin were administered prior to treatment with challenging acute doses of oxycodone and the overall effect of these treatments were shifts of the oxycodone dose response curves to the left, denoting an enhancement. Lorcaserin potentiated the acute antinociceptive effect of oxycodone. In addition, to ensure that this wasn't an oxycodone-specific effect and that it generalized to other opioids, fentanyl and morphine were tested and a similar result was observed where pretreatment with lorcaserin produced a shift of the opioid dose response curve to the left.

The most surprising piece of data is the lack of effect of lorcaserin on methadone-induced antinociception. Methadone is an atypical opioid that possesses a diverse pharmacological profile. Methadone is a high efficacy and long-acting MOR agonist, an NMDA receptor antagonist (which has been implicated in altering the development of opioid tolerance) and is both a serotonin and norepinephrine reuptake inhibitor (Horng *et al.*, 1976; Codd *et al.*, 1995; Ebert *et al.*, 1995; Davis and Inturrisi, 1999; Carpenter *et al.*, 2000; Callahan *et al.*, 2004). The effect of lorcaserin on an opioid could be related to a matter of agonist efficacy, as methadone is reported as a higher efficacy agonist relative to fentanyl, morphine or oxycodone (see Table 1.1 in the introduction) in functional binding studies (Emmerson *et al.*, 1994, 1996; Selley *et al.*, 1997; Alt *et al.*, 1998; Volpe *et al.*, 2011). The additional off-target effects of methadone, namely its NMDA antagonist activity or its reuptake inhibition, are also possible confounds that may prevent any effect of lorcaserin (Ebert *et al.*, 1995; Davis and Inturrisi, 1999; Callahan *et al.*, 2004). Methadone, while an efficacious opioid agonist, is very different from the typical opioids and further studies are necessary to understand why it is not altered by lorcaserin.

It should be noted, however, that the degree to which lorcaserin shifted each of these curves varied. For example, lorcaserin was more efficacious to shift the curve of fentanyl than it was to shift the curve of oxycodone and contrary to both of those compounds, lorcaserin did not alter the dose-response curve of methadone at any dose tested. This could be due to the fact that all opioids present a different pharmacological profile (their efficacies at the MOR) and have different off-target effects (Emmerson *et al.*, 1996; Volpe *et al.*, 2011). Opioid efficacy has been reported previously as a major determinant of drug-drug interactions, specifically in combination with TCAs and SSRIs (Gatch *et al.*, 1998; Banks *et al.*, 2010). A possible mechanism that underlies this difference is the relationship between opioid efficacy and receptor desensitization/internalization (Duttaroy and Yoburn, 1995; Emmerson *et al.*, 1996; Laura M. Bohn *et al.*, 2000; McPherson *et al.*, 2010). High efficacy compounds, such as methadone or DAMGO, readily desensitize and internalize MOR (Keith *et al.*, 1996, 1998; Sternini *et al.*, 1996; Kovoor *et al.*, 1998; Whistler *et al.*, 1999). In contrast, relatively lower efficacy ligands, such as morphine, rapidly desensitize the MOR but are poor at inducing internalization, which is thought to be an underlying component of the tolerance that develops (Keith *et al.*, 1996, 1998; Whistler and von Zastrow, 1998; Lopez-Gimenez *et al.*, 2008; McPherson *et al.*, 2010). Based on these observations and the reports that the effect of serotonergic agents (such as SSRIs) on opioid antinociception are dependent upon opioid efficacy, it is entirely possible that the effect of lorcaserin may similarly be dependent upon MOR agonist efficacy and these effects may be mediated through changes in MOR desensitization and internalization.

Another unusual finding is that the dose-response of lorcaserin to shift the curve of oxycodone was biphasic where 2 mg/kg of lorcaserin produced a greater effect than treating with 4 mg/kg. At high doses, lorcaserin has notable affinity for the 5-HT$_{2A}$ receptor and this receptor, in particular, can function as a “pro-nociceptive” receptor. This observed biphasic effect of lorcaserin and oxycodone could be due to off-target effects mediated through the 5-HT$_{2A}$ receptor. To address this, a transgenic mouse model with a global knockout of the 5-HT$_{2A}$ receptor was utilized. Global knockout of the 5-HT$_{2A}$ receptor trended towards an increase in the potency of morphine (though the ED$_{50}$ were not significantly different). The effect of 5-HT$_{2A}$ receptor knockout on the effect of lorcaserin was inconclusive, as the ED$_{50}$ values from all groups were similar. Lorcaserin in this study did not produce a significant effect on morphine antinociception in the wild-type mice nor did it alter the effect of morphine in the knockout mice. A limitation associated with the 129sV mouse strain is their abnormal responses to opioids, where they exhibit increased opioid-induced locomotor stimulation, increased opioid antinociceptive potency, and a reduced development of tolerance (Crain and Shen, 2000; Murphy *et al.*, 2001). Additional studies may prefer to utilize a mouse strain of the C57/B6J background or some other strain that have not been reported to have abnormal responses to opioids. Therefore, we cannot effectively rule out the contributions of the 5-HT$_{2A}$ receptor in the effect of lorcaserin.

In addition to potentiating the acute antinociceptive effects of oxycodone, it was necessary to evaluate the effect of lorcaserin on time-course of oxycodone’s antinociceptive activity. This was assessed using an ED$_{50}$ dose of oxycodone and two different doses of lorcaserin: a dose that produced a significant potentiating effect (2 mg/kg) and a subthreshold dose that did not significantly shift the oxycodone ED$_{50}$ (0.5 mg/kg) in the warm water tail withdrawal assay. In both cases, lorcaserin enhanced oxycodone’s time course of effect and unsurprisingly, the highest dose of lorcaserin produced the greatest change in overall efficacy and time course of oxycodone. The subthreshold dose, while it did not potentiate the initial antinociceptive effects within the first 15 – 30 minutes, it did demonstrate significant potentiation at the 60-minute time point. These data demonstrate that enhancing oxycodone's time course of effect can be achieved by both a high and a low dose of lorcaserin. The significant potentiation of oxycodone by the low dose is meaningful because subthreshold doses of drugs tend to have fewer dose-dependent side effects.

After evaluating the effect of lorcaserin via subcutaneous administration and considering the previous data demonstrating the site-specific effect of lorcaserin, further studies evaluated a possible locus for a potential opioid/lorcaserin interaction. The hypothesis that lorcaserin produced its potentiating effect within the spinal cord was developed after observing the differential activity of lorcaserin alone in the brain and the spinal cord. This hypothesis was tested using a subthreshold dose of intrathecal lorcaserin (a dose which did not produce a statistically significant effect in the warm water tail withdrawal) and an ED_{50} dose of oxycodone. The combination produced a roughly ~40% increase in the antinociceptive effect relative to oxycodone alone but failed to reach statistical significance (P-value = 0.06). Although not statistically significant, these data suggest that the effect is at least partially mediated at this spinal level but may also require the addition of peripheral cell bodies such as dorsal root ganglion neurons (which will be discussed further on) and higher order spinal structures or cortical brain regions. Nonetheless, the combined enhanced effect of oxycodone and intrathecal lorcaserin are supported by the observation that both MOR and 5-HT_{2C} are expressed in the dorsal horns of the spinal cord (Clemett, *et al.*, 2000; Millan, 2002). It is unclear if they are colocalized on the same neurons but studies suggest that the 5-HT_{2C} receptor may be expressed on GABAergic interneurons and may act through an "excitation of inhibition" (Di Matteo *et al.*, 2000; Di Giovanni *et al.*, 2001; Giorgetti and Tecott, 2004; Bubar and Cunningham, 2007; Theile *et al.*, 2009; Bubar *et al.*, 2011). The limitation of this assumption, however, is that the expression of the 5-HT$_{2C}$ receptors on GABAergic neurons was characterized in regions linked to drug abuse, such as the ventral tegmental area, and is has yet to be elucidated if this pattern of expression extends to other physiological systems such as antinociception (Di Matteo *et al.*, 2000; Di Giovanni *et al.*, 2001; Giorgetti and Tecott, 2004; Bubar and Cunningham, 2007; Theile *et al.*, 2009; Bubar *et al.*, 2011).

Alternatively, there is another common link between the serotonergic system and the opioidergic system: noradrenaline (Ossipov *et al.*, 1985; Cui *et al.*, 1999; Fairbanks and Wilcox, 1999; L M Bohn *et al.*, 2000; Fairbanks *et al.*, 2002). Opioids have been shown to stimulate the release of noradrenaline in the spinal cord and this action may, in part, underlie their antinociceptive effects (Bouaziz *et al.*, 1996; Cui *et al.*, 1999; Millan, 2002). In addition, 5-HT$_{2C}$ receptor agonists are shown to stimulate the release of noradrenaline and their antinociceptive effects are antagonized by administration of yohimbine, an α_2-adrenoreceptor antagonist(Obata *et al.*, 2007). The interactions between opioids and 5-HT$_{2C}$ receptor agonists, like lorcaserin, may be the result of a "sum-of-the-parts" mechanism whereby cumulative interactions with many neurotransmitter systems yield an overall enhanced antinociceptive effect. The neurobiology regarding pain is incredibly complex and neurotransmitter systems exhibit varied "cross-talk" and it is possible that these interactions are not purely MOR/5-HT$_{2C}$ receptor mediated.

In addition to evaluating the intrathecal lorcaserin/oral oxycodone interactions, it was of interest to evaluate intracerebroventricular lorcaserin and oral oxycodone administration. The lack of effect of lorcaserin when administered intracerebroventricularly was unexpected and its combination with oxycodone, significantly attenuated the antinociceptive effects of oxycodone. This observation was unexpected, as it further suggests that the 5-HT$_{2C}$ receptor serves a differential role in the spinal cord and in supraspinal structures. The attenuation, however, could be attributed to changes in body temperature, as the warm-water tail withdrawal test has been noted to be sensitive to such physiological states (Tjolsen and Hole, 1993). 5-HT$_{2C}$ receptor agonists, in particular, are thermogenic, in that they raise overall body temperature (Hayashi *et al.*, 2004). One hypothesis to explain the observed effect is that intracerebroventricular administration of lorcaserin, due to its proximity to the hypothalamus, raised core body temperature which would alter the observed antinociceptive effect of an opioid (Hayashi *et al.*, 2004).

An additional alternative explanation for the i.c.v. lorcaserin/oral oxycodone results is that perhaps the 5-HT$_{2C}$ receptor serves differential roles in the higher order brain structures and in the spinal cord. Several studies have shown that spinal 5-HT$_{2C}$ receptors serve an antinociceptive role by administration of several different 5-HT$_{2C}$ agonists in models of neuropathy and chronic pain (Obata *et al.*, 2003, 2004, 2007; Nakai *et al.*, 2010) and the data reported here supports these observations where intrathecal lorcaserin was antinociceptive in acute pain. 5-HT$_{2C}$ receptor activity in the amygdala is implicated in the inefficacy of SSRIs in the treatment of neuropathy and two studies have demonstrated that genetic knockdown or site-specific administration of a 5-HT$_{2C}$ receptor antagonist inhibits nocifensive behaviors from rodents and improves analgesic efficacy of SSRIs (Grégoire and Neugebauer, 2013; Ji *et al.*, 2017). This supports the finding observed where intracerebroventricular lorcaserin is capable of attenuating the antinociceptive effect of oxycodone. These studies support the hypothesis that the 5-HT$_{2C}$ receptor in supraspinal regions serves a different role and may be “pro-nociceptive”.

Following the studies on the acute interactions between lorcaserin and oxycodone, it became clear that it was necessary to evaluate the effect of lorcaserin on chronic opioid treatment and tolerance. It was important to evaluate the effect of lorcaserin on opioid tolerance because there are very rare cases in which a patient takes only one dose of an opioid. Opioid tolerance is a challenge in that it is commonly managed through dose escalation and incidentally high doses of opioid are associated with increased mortality (Dasgupta *et al.*, 2015; Dowell *et al.*, 2016a; Shah *et al.*, 2017).

There are several major findings that suggest that alterations in opioid tolerance by lorcaserin may be underlying the observed acute interactions discussed in Chapter 2. First, acute dose-response curves were generated using a cumulative dosing paradigm which occurs over a period of several hours. Following activation of a receptor by an agonist, rapid desensitization occurs within seconds to minutes of exposure and it is reasonable to hypothesize that lorcaserin may be altering MOR desensitization and this underlies the ED_{50} shifts that were observed (Stadel *et al.*, 1983; Sibley *et al.*, 1987; Kovoor *et al.*, 1998; Alvarez *et al.*, 2002; Borgland *et al.*, 2003; Williams *et al.*, 2013). The changes in the time course of oxycodone's activity also supports this hypothesis, as its effects are significantly enhanced 1 – 2 hours post administration.

There are several means through which tolerance can be induced. In these studies, we opted to evaluate two models of tolerance, with the idea being that it will provide information on the mechanism through which lorcaserin is acting. The time scale for acute tolerance is generally very rapid and occurs within minutes to hours after drug exposure. It is characterized by a rapid desensitization of the receptor and that involves eventual endocytosis, possible receptor recycling, and this leads to the expression of what is observed as "acute" tolerance (Cox et al., 1968; Huidobro-Toro and Way, 1978; Ling et al., 1989; Fairbanks and Wilcox, 1997; Bohn et al., 2000). For the purpose of these studies, acute tolerance is defined as a single drug administration or series of drug exposures that is confined to one day. Chronic (long-term) models of tolerance occur on a timescale of days to week and are typically encompassed by repeated drug administrations. The mechanisms underlying this process are not as well understood but are thought to involve multiple regulatory mechanisms, such as changes in intracellular signaling cascades (e.g., cAMP upregulation) and receptor downregulation (Tempel and Zukin, 1987; Tempel, 1991; Tao *et al.*, 1993; Z Wang *et al.*, 1994; Wang *et al.*, 2004; Sim-Selley, 2005; Shoblock and Maidment, 2006; Sim-Selley *et al.*, 2009). Use of these paradigms allow us to understand the contributions of lorcaserin, and by extension the effect of activation of the 5-HT$_{2C}$ receptor, in the regulation of the MOR with repeated opioid administrations. In addition, studies evaluating the effect of lorcaserin on the single-cell level and at the receptor level were conducted in addition to the *in vivo* studies because it provides a greater understanding of how lorcaserin is altering opioid activity. Collectively, these studies provide a greater understanding of opioid tolerance and suggest a possible role of serotonergic mechanisms and 5-HT$_{2C}$ receptor ligands as a means to alter the antinociceptive effects of opioids in a manner that is beneficial to the patient.

The initial studies using the tolerance model where animals received only a single high dose of oxycodone (100 mg/kg, p.o.) prior to the challenge dose the following day demonstrated that lorcaserin was capable of completing blocking the development of acute tolerance. It is interesting to note that although the lorcaserin treatment on its own did not produce a statistically significant effect relative to vehicle controls, the overall antinociceptive response after the challenge dose was considerably higher. This same pattern was observed in animals that received the high oxycodone and lorcaserin pretreatments where the overall antinociceptive effect was modestly greater than the vehicle controls but not statistically significant.

Based on the data presented in Chapter 2, the current hypothesis is that lorcaserin is altering the antinociceptive effects by way of the spinal cord (even when administered subcutaneously and not intrathecally), there are additional structures that can provide insight into this interaction.

Dorsal root ganglion (DRG) neurons are peripherally located structures that are comprised of afferent nerve fibers that synapse in the dorsal horn of the spinal cord. Though technically outside of the central nervous system, previous studies have demonstrated their role in the development of opioid tolerance (Corder *et al.*, 2017; Jacob *et al.*, 2018). This site is of particular interest in our studies because DRGs are a critical component of the nociceptive circuitry and a well-validated model for evaluating cellular tolerance (Gracious R. Ross *et al.*, 2012; Kang *et al.*, 2017; Jacob *et al.*, 2018). Incubating DRG neurons overnight in oxycodone produces reproducible tolerance and with this model, the effect of lorcaserin on overnight exposure to oxycodone was assessed. These studies demonstrated that co-incubation of lorcaserin and oxycodone attenuated the development of acute tolerance on a single-cell level. This effect corroborates nicely with the observed effect *in vivo* and suggest that within the time course of one day, lorcaserin can significantly attenuate acute tolerance.

The longer-term, or multiple day treatment, model of tolerance consisted of twice daily treatments for four days with oral oxycodone and/or lorcaserin treatments. The mechanisms that underlie this process are considered to be distinct from that which involves the development of acute tolerance (as reviewed by Williams *et al.*, 2013). The observation that lorcaserin only partially attenuated the development of tolerance in the long-term model suggests that the mechanisms through which 5-HT$_{2C}$ receptor activation alters opioid tolerance differs based on the frequency and timing of opioid administration. The effects in mice that were treated with both lorcaserin and oxycodone were significantly different from both tolerant (treated with only chronic oxycodone) and vehicle control mice in terms of their antinociceptive responsivity to the oxycodone challenges. These data and the acute tolerance data suggest that the mechanisms through which the activation of the 5-HT_{2C} receptor work are distinct from each other because lorcaserin did not equivalently block the development of tolerance in the two models.

It is now well accepted that at a cellular level, adaptive changes occur following chronic opioid administration and these changes are marked by changes in G-protein-coupled receptor function (Tao *et al.*, 1993; Sim *et al.*, 1996; Bernstein and Welch, 1998; Sim-Selley, 2005; Priyanka A. Madia *et al.*, 2012; Arttamangkul *et al.*, 2018). Receptor desensitization is characterized by uncoupling of the receptor from the G-protein (also known as desensitization which is mentioned earlier) and eventual internalization of the receptor leading to longer-term receptor recycling and downregulation (Law *et al.*, 1984; Tempel *et al.*, 1988; Tempel, 1991; Ferguson *et al.*, 1996; Ronnekleiv *et al.*, 1996; Kovoor *et al.*, 1998; Alvarez *et al.*, 2002; Arttamangkul *et al.*, 2008; Lopez-Gimenez *et al.*, 2008; Priyanka A. Madia *et al.*, 2012; Williams *et al.*, 2013). Functional activity of these receptors can be assessed using agonist-stimulated [^{35}S]GTPγS binding. Changes in MOR activity following chronic opioid treatment is noted in several brain regions and the spinal cord (Sim *et al.*, 1996; Sim-Selley, 2005; Sim-Selley *et al.*, 2009). Our hypothesis was to test if chronic treatment with both lorcaserin and oxycodone altered the functional activity of the MOR and allowed it to signal in a manner similar to naïve controls.

The binding data demonstrate that treatment with lorcaserin did not block reductions in MOR functional activity following treatment with chronic oxycodone. Chronic oxycodone alone reduced basal activity (binding the absence of an agonist) and this effect was modestly restored by co-treatment with lorcaserin but overall maximal binding between the two groups was not significantly different. In addition, chronic treatment with lorcaserin alone did not alter DAMGO-stimulated binding which agrees with the *in vivo* results where chronic lorcaserin did not significantly shift the ED_{50} of acute oxycodone relative to vehicle controls. Overall, these data suggest that the effect of lorcaserin on opioid tolerance is not mediated through changes in functional activity at the MOR and is in fact working through some other mechanism that remains to be elucidated. The evidence presented here suggests that the effect of lorcaserin could be due to activation of the 5-HT$_{2C}$ receptor rather than alterations in the regulation of the MOR.

Alternative explanations for the changes in basal activity, however, could be attributed to differences in MOR downregulation between groups. [^{35}S]GTPγS studies do not assess receptor densities and it is possible that lorcaserin may be altering receptor expression levels. It has also been noted that in addition to opioid tolerance, tolerance to the effects of lorcaserin may develop following its repeated administration (Van Oekelen *et al.*, 2003). It is hypothesized that expression of the 5-HT$_{2C}$ receptor is inducible following injury, such an inflammatory pain states, but limited data is available exploring the relationship between chronic opioid administration and 5-HT$_{2C}$ expression (Wu *et al.*, 2001; Liu *et al.*, 2005).

Further investigations into the interactions between the opioids and the 5-HT$_{2C}$ receptor are important for several reasons. Opioids are reported to stimulate the release of serotonin and with chronic administration of opioids, there are alterations in serotonin synthesis and turnover that is observable *in vivo* (Theiss *et al.*, 1975; Yaksh and Tyce, 1979). Under conditions of sustained serotonin depletion, the 5-HT$_{2C}$ receptor undergoes pre-mRNA transcript editing that allows for the synthesis of a 5-HT$_{2C}$ receptor isoform with greater affinity for serotonin and additionally increases the expression of the 5-HT$_{2C}$ receptor (Fitzgerald *et al.*, 1999; Gurevich *et al.*, 2002; Schmauss, 2005). Sustained changes in neurotransmission following chronic opioid exposure may have the capacity to alter the activity of the 5-HT$_{2C}$ receptor (Zhang *et al.*, 2015). For example, chronic morphine treatment is reported to increase 5-HT$_{2C}$ receptor protein expression in the nucleus accumbens, locus coeruleus, and ventral tegmental area (Zhang *et al.*, 2015). These data suggest that opioid treatment may alter 5-HT$_{2C}$ receptor functionality and these changes need further investigation.

Current Opioid-Sparing Adjuncts and Lorcaserin

There are many current opioid-sparing treatment options for the treatment of pain, including NSAIDs, acetaminophen, α_2-receptor agonists, NMDA antagonists, and antidepressants. Each class displays specific efficacy in treating certain types of pain. NSAIDs and acetaminophen are useful for treating acute and post-operative pain (Cassinelli *et al.*, 2008; Derry *et al.*, 2009, 2013; Gaskell *et al.*, 2009). Limitations of NSAID and acetaminophen use are marked by an increased risk of adverse gastrointestinal effects and potentially fatal hepatoxicity, respectively (Laine, 2002, 2003; James *et al.*, 2003; Bhala *et al.*, 2013).

Agonists at the α_2-adrenergic receptor have demonstrated remarkable synergism with opioids in preclinical studies but their efficacy in humans is debated (Benhamou *et al.*, 1994; Fairbanks and Wilcox, 1999; Fairbanks *et al.*, 2002; Özdoğan *et al.*, 2003; Blaudszun *et al.*, 2012; Stone *et al.*, 2014). A major clinical concern for use of clonidine is the risk of hypotension and bradycardia and this combination may be risky for hemodynamically unstable patients but additional studies suggest that this risk can be minimized by titrating the dose of α_2-receptor agonist (Ebert *et al.*, 2000; Hall *et al.*, 2000; Stone *et al.*, 2014).

NMDA antagonists, such as ketamine, is a safe and effective opioid-sparing adjunct but the potential for hallucinogenic side effects limits its use to in-patient settings where patients can be closely monitored by attending nurses and physicians (Yamauchi *et al.*, 2008; Laskowski *et al.*, 2011; Brinck *et al.*, 2017). NMDA antagonists are also shown in preclinical studies to block the development of opioid tolerance (Trujillo and Akil, 1991; Tiseo *et al.*, 1993; Elliott *et al.*, 1994). Overall, NMDA antagonists have a favorable opioid-sparing profile and the potential for psychomimetic effects can be mitigated by administration of sub-dissociative doses(Yamauchi *et al.*, 2008; Laskowski *et al.*, 2011; Miller *et al.*, 2015; Motov *et al.*, 2015; Brinck *et al.*, 2017).

Antidepressants, such as SSRIs and TCAs, are routinely used as first-line pharmacotherapuetics for the treatment of chronic pain conditions in spite of their highly debated efficacy (Watson, 2000; Dworkin *et al.*, 2007; Saarto and Wiffen, 2007; Moore *et al.*, 2015; Welsch *et al.*, 2018). Studies evaluating the use of antidepressants in the treatment of acute and post-operative pain lack sufficient evidence and require further investigation but current data suggests they may have some utility (Wong *et al.*, 2014; Gilron, 2016). The major risk associated with antidepressant use if the risk of serotonin syndrome and this risk may be greater in combination with an opioid (Boyer and Shannon, 2005; Gillman, 2005; Sansone and Sansone, 2009; Rastogi *et al.*, 2011).

Additional opioid-sparing agents are described in Table 4.1. Each class presents its own set of benefits and potential risk. The major conclusion that can derived is that there are specific cases in which certain treatments may be preferable. For example, for the treatment of chronic pain, use of an antidepressant or gabapentin would be favorable to treatment with an NSAID, and then treatment for acute pain would likely use NSAIDs or acetaminophen. Lorcaserin and oxycodone are a potentially useful combination because it alters both the acute and the chronic effects of opioids which few combinations achieve. The studies in this dissertation provide evidence of lorcaserin’s utility as an opioid-sparing treatment for acute pain. In cases where hepatoxicity or gastrointestinal bleeding are of concern, lorcaserin and oxycodone may be preferable to NSAIDs or acetaminophen. Obviously further studies of the risks are necessary though before any significant conclusions of its clinical utility can be made.

Translational considerations for 5-HT_{2C} agonists and lorcaserin.

Sex differences are an important consideration in the translation of a potential pharmacotherapeutic treatment into the clinic. There are significant sex differences in the pain severity and frequency in clinical populations, where women frequently report more pain than their male counterparts (Attanasio and Andrasik, 1987; Henry *et al.*, 1992; Pietri *et al.*, 1992; Unruh, 1996; Aubrun *et al.*, 2005). In preclinical studies, treatment with morphine also displays similar sex differences, where morphine is generally more potent in male than in females and males develop greater tolerance following repeated opioid administration (Kepler *et al.*, 1991; Bartok and Craft, 1997; Craft *et al.*, 1999; Mogil *et al.*, 2000). This observed difference in the pharmacodynamic effects also generalizes to human studies, where women experienced greater levels of post-operative pain and received more frequent morphine administrations (Aubrun *et al.*, 2005).

This dissertation has discussed the importance of serotonin in the physiology of pain and it should come as no surprise that there are also significant sex differences in the concentrations of brain serotonin and serotonin transporter function (Nishizawa *et al.*, 1997; Zhang *et al.*, 1999; Cannon *et al.*, 2013). The 5-HT_{2C} receptor displays a sex-specific polymorphism that is associated with impaired functionality (Fehr *et al.*, 2000; Anastasio *et al.*, 2014). It could be hypothesized that lorcaserin may have reduced efficacy in female populations, assuming that they possess the 5-HT_{2C} receptor polymorphism. Overall, the significant differences in serotonin physiology in females and the significant differences in the potency of morphine in females makes it difficult to speculate the potential efficacy of lorcaserin in these populations without further testing.

Figure 4.1: Comparison of opioid-sparing adjunctive therapies. Agents which alter neurotransmission, agonists and antagonists of the cholinergic, GABAergic, and other neurotransmitter systems, have also been shown to alter the potency and tolerance to opiates in laboratory animals. Obviously, additional work is needed before a novel compound can be proposed to be an opioid-sparing agent in man. (make a footnote)

Drug Class	**Compounds**	**Opioid-sparing?**	**Alters the chronic effects of opioids?**	**Other considerations?**	**Reference**
NSAIDs	Ibuprofen Ketorolac Celecoxib Naproxen Diclofenac	Yes, in both acute and chronic pain.	In preclinical studies, intrathecal administration reverses tolerance	Risk of adverse GI effects.	(Malmberg and Yaksh, 1993; Powell *et al.*, 1999; Wong *et al.*, 2000; Kolesnikov *et al.*, 2003; Huang *et al.*, 2008; Derry *et al.*, 2009, 2013)
Acetaminophen		Yes, in both acute and chronic pain.	n/a	Hepatoxicity	(Sunshine *et al.*, 1993; Raffa *et al.*, 2000; James *et al.*, 2003)
NMDA Antagonists	Ketamine	Maybe. The opioid-sparing effect is controversial.	Yes. Possibly blocks the development of tolerance.	Psychomimetic effects & hallucinations with large doses.	(Brinck *et al.*, n.d.; Trujillo and Akil, 1991; Elliott *et al.*, 1994; Jaksch *et al.*, 2002; Yamauchi *et al.*, 2008; Laskowski *et al.*, 2011)
Anticonvulsants	Gabapentin Pregabalin	Debatable in acute pain. Efficacy in treating chronic pain. Potentiates acute opioid antinociception.	Yes. Can alter the development of tolerance and reverse tolerance.	Gabapentenoids & opioids may be a fatal combination. Less efficacious than NSAIDs/acetaminophen.	(Biederman *et al.*, 2003; Straube *et al.*, 2010; Aguado *et al.*, 2012; Wibbenmeyer *et al.*, 2014; Lyndon *et al.*, 2017)
Antidepressants	TCAs SSRIs	Yes, in preclinical studies. Effective for chronic pain. Limited studies in acute pain.	Maybe. TCAs attenuate morphine tolerance. SSRIs need further study.	Serotonin syndrome.	(Larson and Takemori, 1977; Kellstein *et al.*, 1984; Hynes *et al.*, 1985; Banks *et al.*, 2010; Li *et al.*, 2011(Larson and Takemori, 1977; Tai *et al.*, 2007; Huang *et al.*, 2012))
Cannabinoids	Δ^9-THC	Yes, in preclinical studies. Needs further testing in humans.	Yes, may block the development of tolerance.	Psychoactive effects, tolerance, and also illegal.	(Welch *et al.*, 1995; Cichewicz and McCarthy, 2003; Naef *et al.*, 2003; Smith *et al.*, 2007; Nielsen *et al.*, 2017)
α_2 agonists	Clonidine	Yes, in preclinical studies but debatable efficacy in humans.	Maybe. Needs further study.	Risks of hypotension and bradycardia	(Malmberg and Yaksh, 1993; Benhamou *et al.*, 1994; Fairbanks and Wilcox, 1999; Özdoğan *et al.*, 2003; Gursoy *et al.*, 2011; Blaudszun *et al.*, 2012; Stone *et al.*, 2014)
5-HT$_{2C}$ Agonist	Lorcaserin	Yes, in preclinical studies. Needs further study in humans.	Yes, may block the development of tolerance.	Debated efficacy in attenuating abuse-related effects.	(Neelakantan *et al.*, 2017)

A final consideration is the possibility of serotonin syndrome with combined treatment of an opioid and lorcaserin. Opioids are reported to alter the kinetics of serotonin and this may lead to an increased risk of serotonin syndrome (Raffaello *et al.*, 1975; Theiss *et al.*, 1975; Gillman, 2005; Sansone and Sansone, 2009; Rastogi *et al.*, 2011). Serotonin syndrome, or serotonin toxicity, is a collection of symptoms that includes: changes in cognition, autonomic hyperactivity, and neuromuscular abnormalities, in addition to other symptoms such as tremor, diarrhea, neuromuscular rigidity and hyperthermia (Boyer and Shannon, 2005). The 5-HT$_2$ receptor family is implicated as a mediator of some serotonin syndrome symptoms and in a preclinical model of serotonin syndrome, the behaviors were antagonized by administration of a 5-HT$_{2A}$ antagonist or a 5-HT$_{2B/2C}$ antagonist (Van Oekelen *et al.*, 2002). In the case of lorcaserin and oxycodone, the incidence of serotonin syndrome may be mitigated by the use of a lower dose of oxycodone and a low dose of lorcaserin. There is a lack of studies that have specifically investigated the contributions of the 5-HT$_{2C}$ receptor in the pathogenesis of serotonin syndrome so additional studies investigating its role are needed. Overall, the risk of serotonin syndrome in the combined treatment of lorcaserin and oxycodone is not clear. Anecdotally, animals that were treated with oxycodone and lorcaserin (in the described studies in this dissertation) did not display any signs of serotonin-syndrome behaviors (forepaw treading, resting tremor, rigidity, Straub tail, hind limb abduction, and head weaving) (Haberzettl *et al.*, 2013). Nonetheless, it is important to stress the importance of specifically evaluating this potential interaction with further study because it may be a potential clinical limitation.

Final Conclusions

Overall, our studies suggest an opioid-sparing role for lorcaserin and a possible time-dependent mechanism through which it may be working through. As implicated by the acute studies with a combination of lorcaserin and oxycodone, an overall lower dose of opioid is necessary to induce an antinociceptive effect. Furthermore, the idea that tolerance can be avoided by treatment with a low dose combination may be attainable (Smith *et al.*, 2007). Although lorcaserin did not fully attenuate tolerance in the long-term model, perhaps if a lower dose of oxycodone were used with lorcaserin, the development of tolerance may be abrogated completely.

Collectively, the results from these experiments in this dissertation further expand our understanding of the interactions between opioids and the 5-HT$_{2C}$ receptor in both acute administration and tolerance. Acute interactions between lorcaserin and several opioids, mainly oxycodone, were thoroughly characterized *in vivo* and showed that lorcaserin, and another 5-HT2$_{2C}$ receptor agonist, potentiate their acute antinociceptive effects through activation of the 5-HT$_{2C}$ receptor and not the 5-HT$_{2A}$ receptor. Furthermore, our data show that these effects are not mediated through changes in opioid metabolism, as lorcaserin did not have an effect on the distribution or metabolism of oxycodone at all time points evaluated. From those studies, we evaluated another important component of opioid pharmacology, opioid pharmacology, and found that lorcaserin had differential effects. In a model of short-term, acute, tolerance both *in vivo* and *in vitro*, lorcaserin completely attenuated the development of tolerance but in a longer-term model of tolerance (using the whole animal approach and the binding studies), lorcaserin only partially attenuated tolerance development. These data suggest that the mechanisms that underlie these two stages of tolerance are distinct and that the activation of 5-HT$_{2C}$ receptor plays a differential role in both phases. As an opioid-sparing combination, lorcaserin may be useful as it enhances the acute effects (and thus reduces the required dose of opioid needed) and alters the development of tolerance with chronic use (which can also be mitigated by an overall lower dose of oxycodone consumed). Additionally, these studies provide some insight into the mechanisms through which lorcaserin is producing its opioid-sparing effects and are hypothesized to comprise spinally-mediated mechanisms. These studies demonstrate that serotonergic mechanisms, particularly those that involve the 5-HT$_{2C}$ receptor, may be a useful avenue for further investigation in the development of alternative opioid-sparing therapeutics.

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