OPIOID ANTINOCICEPTION AND ANTIHYPERALGESIA IN COMBINATION WITH METABOTROPIC GLUTAMATE RECEPTOR ANTAGONISM

Dana Elaine Daugherty

A thesis submitted to the faculty of the University of North Carolina at Chapel Hill in partial fulfillment for the requirements for the degree of Master of Arts in the Department of Psychology.

Chapel Hill
2010

Approved by:

Linda Dykstra, Ph.D.

Mitchell Picker, Ph.D.

Mark Hollins, Ph.D.

ABSTRACT

DANA DAUGHERTY: Opioid Antinociception and Antihyperalgesia in Combination with Metabotropic Glutamate Receptor Antagonism
(Under the direction of Linda Dykstra, Ph.D.)

Research has shown that the analgesic effects of mu-opioids can be enhanced by concurrent administration of drugs that target various sites within the glutamate system. To explore these effects, the present study examined the effects of a metabotropic glutamate receptor subtype 1 (mGluR1) antagonist and a metabotropic glutamate receptor subtype 5 (mGluR5) antagonist in combination with morphine. These interactions were examined in two models of acute pain and one model of inflammatory pain. Selected doses of the mGluR1 antagonist JNJ16259685 (1.0 – 5.6 mg/kg) and the mGluR5 antagonist MPEP (30.0 mg/kg) enhanced morphine antinociception in both models of acute pain. Neither antagonist enhanced morphine-antihyperalgesia in the model of inflammatory pain; however, MPEP (10.0 mg/kg) did attenuate the antihyperalgesic effects of high doses of morphine. These results suggest that the interaction between the mGluR and opioid receptor systems depends on the assay used to examine these effects and the dose combinations examined.

TABLE OF CONTENTS

LIST OF FIGURES

Figure

Appendix

CHAPTER 1

INTRODUCTION

Literature Review

Pharmacological agonists of the endogenous opiate system produce both reliable analgesia and an array of side effects. The most relevant clinical opioid compound, morphine, binds to and activates the family of mu (μ)-opiate receptors (MORs) with high affinity (Nishimura et al., 1984). Endogenous activation of the MOR by opiates such as endorphins and enkephalins results in mild analgesia (Belluzi et al., 1976; Hughes et al., 1975). Morphine mimics this activation but with higher efficacy (Pasternak, 1981), which has made it a standard in clinical pain management; however, morphine's analgesic use is often limited by a number of undesirable side effects.

The side effects of morphine and other μ-opioid agonists can include, among others, respiratory depression, decreased blood pressure and heart rate, nausea, and constipation (Caligano et al., 1991; Trescot et al., 2008). In addition to these effects, repeated use of morphine carries additional risks. For example, physical dependence is a common complication of repeated opioid exposure. In addition to physical dependence, repeated exposure to morphine and other μ-

opioids can result in tolerance (Koch & Hollt, 2008), in which a higher dose of the drug is needed to achieve the same effect previously produced by a lower dose.

Tolerance to the analgesic effects of morphine and other μ-opioid agonist compounds is a great concern for many clinicians and preclinical researchers. While increasing the morphine dose in a tolerant individual will produce sufficient analgesia, the increase in dose may increase the occurrence of morphine's other effects, such as respiratory depression. Even in response to acute dosing, it can be difficult to balance morphine's analgesic efficacy with its side effect profile. For this reason, many preclinical researchers have turned their efforts to enhancing the analgesic effects of morphine and other opioid compounds without also increasing the many side effects of this pharmacological class.

The excitatory amino acid glutamate plays a facilitatory role in pain processing. Glutamatergic inputs from afferent nociceptive fibers carry pain signaling into the central nervous system (Krarup, 2003). If this type of glutamate signaling could be blocked during opioid treatment for pain, the administered opioid may display greater analgesic efficacy. With this in mind, researchers from our laboratory and others' have focused their efforts on the blockade of a widely distributed, ionotropic glutamate receptor, the NMDA receptor, in combination with opioid agonist treatment.

Preclinical research conducted on the antinociceptive efficacy of opioid agonist/NMDA antagonist combinations indicates that antinociception can be enhanced with these combinations. Fischer et al. (2005) found that an NMDA antagonist enhanced the acute antinociceptive effect of morphine in the tail-flick

test of thermal nociception, an effect also seen with lower efficacy opioids (Fischer et al., 2006). A large number of preclinical studies have found that NMDA antagonism can enhance opioid analgesia in mouse models of acute pain (Fischer et al., 2005; Fischer et al., 2006), in rat models of chronic pain (Lomas et al., 2008; Henry et al., 2008), and in non-human primate studies of pain and analgesia (Allen & Dykstra, 2001). Chen et al. (2005) reported an enhancement of morphine antinociception by the NMDA antagonist dextromethorphan, but an attenuation of acute codeine antinociception, suggesting an interaction between the two receptor systems. An interaction between the two systems is further supported by the findings of Nemanni et al. (2004), in which NMDA antagonism enhanced low-dose morphine antinociception but attenuated high-dose morphine antinociception. Furthermore, in addition to its effects on the acute antinociceptive effects of MOR agonist compounds, NMDA antagonism also prevents the development of tolerance to the antinociceptive effects of morphine (Allen & Dykstra, 2000; Kozela et al., 2003; Trujillo & Akil, 1991) in rodents and primates. Moreover, some NMDA antagonists decrease physical dependence associated with chronic morphine exposure (Trujillo & Akil, 1991).

Taken together, preclinical studies of NMDA antagonists in combination with morphine and other opioid compounds show promise for enhancing clinical analgesia; however, clinical studies reveal mixed results. For example, whereas Bossard et al. (2002) found that the NMDA antagonist ketamine enhanced morphine analgesia, a similar study by Galer et al. (2005) with the NMDA antagonist dextromethorphan did not reveal a similar effect. Additionally, clinical

reports of side effects such as nausea, dizziness, and fatigue limit the use of these combinations. In much the same way that the gastrointestinal side effects of MOR agonists are due to the receptor's prevalence in the gastrointestinal tract, the widespread anatomical distribution and functionality of the NMDA receptor may make its pharmacological targeting produce a range of side effects.

Given the limitations of NMDA antagonist treatment, many preclinical researchers are studying the roles of other glutamate receptors in pain regulation. The Group I family of the metabotropic class of glutamate receptors is particularly interesting in this respect. The metabotropic glutamate receptor subtypes 1 (mGluR1) and 5 (mGluR5) are post-synaptic receptors which are highly expressed in areas of the pain processing system, most notably on peripheral afferent nociceptive fibers (Walker et al., 2001b; Young et al., 1997) and in the dorsal horn neurons of the spinal cord (Alvarez et al., 2000; Jia et al., 1999; Pitcher et al., 2007). Given the high expression of mGluR1 and mGluR5 in peripheral nociceptive fibers and in the dorsal horn, an area which receives nociceptive input from peripheral fibers and carries that input further into parts of the central nervous system densely populated with MORs, it is interesting to examine interactions between opioid analgesics and mGluR antagonists.

The growing body of literature on Group I mGluR antagonist/μ-opioid combinations implicates the mGluR1 receptor in modulation of pain processing and opioid analgesia in acute pain states while the mGluR5 receptor has been implicated in modulation of pain processing and opioid analgesia in chronic and inflammatory pain states. Research utilizing preclinical models of acute pain

(clinical pain states that typically result from a known, short-lived noxious input, such as post-surgical pain) has demonstrated that mGluR1 antagonism can produce antinociceptive effects when administered alone (Neugbauer et al., 1999) and can enhance the acute antinociceptive effects of morphine and other μ-opioids (Fischer et al., 2008a; Fischer et al., 2008b). Interestingly, the same mGluR1 antagonist (JNJ16259685) used in the Fischer et al. studies was also examined in a model of chronic pain and did not enhance morphine analgesia (Henry et al., 2008). These data support the hypothesis that the mGluR1 site is an important modulator of acute pain processing and analgesia.

Studies of chronic pain states (clinical pain states that typically result from sustained inflammatory input that can actually alter the functionality of the pain processing system), however, indicate that mGluR5 antagonists can enhance morphine's analgesic effects. Studies employing inflammatory models of chronic hyperalgesia and allodynia have shown enhancement of morphine antihyperalgesia by mGluR1/5 antagonists (Fisher & Coderre, 1996) and selective mGluR5 antagonists (Gabra et al., 2008; Kozela et al., 2003). Furthermore, some mGluR5 antagonists produce antihyperalgesic effects when administered alone in inflammatory models (Lee et al., 2006; Walker et al., 2001a; Walker et al., 2001b) and inhibit the development of tolerance to the antinociceptive effects of morphine following chronic morphine exposure (Gabra et al., 2008; Kozela et al., 2003; Smith et al., 2004). Furthermore, the mGluR5 receptor has been shown to up-regulate in response to persistent inflammation (Dolan et al., 2003) and in response to post-surgical pain (Dolan et al., 2004).

While the literature seems to point to differential roles between mGluR1 and mGluR5 in pain and analgesia modulation, there have been no published studies to date that have examined both mGluR1 and mGluR5 antagonists in both acute and inflammatory/chronic pain models within a single animal species. Differences across published studies include species studied (i.e., mouse vs. rat), type of pain modeled (i.e., acute vs. chronic), and mGluR subtype targeted (i.e., mGluR1 vs. mGluR5), which makes it difficult to reach conclusions regarding the roles of each receptor in pain and opioid modulation. Furthermore, there are occasional reports in the literature of mGluR1 antagonism producing analgesic effects in chronic pain models. For example, the mGluR1 antagonist CPCCOEt decreased nociceptive behaviors in rats with chronic constriction nerve injuries and Complete Freund's Adjuvant pretreatment (Kumar et al., 2010), and antisense oligonucleotide knockdown of mGluR1 resulted in attenuation of cold-, heat-, and mechanical-allodynia in neuropathic rats (Fundytus et al., 2001). These cases cannot be ignored, but, again, without a full examination of both receptor types in both types of pain models within a single animal species, conclusions are difficult to reach. Therefore, the present study was designed to examine the effects of selective mGluR1 antagonism and selective mGluR5 antagonism alone and in combination with μ-opioid analgesia in models of both acute pain and inflammatory/chronic pain in a single animal species.

Experimental Aims

The present study examined μ-opioid analgesics in combination with the mGluR1 antagonist JNJ16259685 ([(3,4-dihydro-2H-pyrano[2,3-b]quinolin-7-yl)-(cis-4-methoxycyclohexl)-methanone]) and the mGluR5 antagonist MPEP (2-methyl-6-(phenyl-ethynyl) pyridine hydrochloride) in the rat in models of both acute and inflammatory pain. The mGluR1 antagonist JNJ16259685 (JNJ) was chosen for its relatively high selectivity for the mGlu1 receptor (Lavreysen et al., 2004). The mGluR5 antagonist MPEP was likewise chosen for its mGlu5 receptor selectivity (Gasparini et al., 1999) and for its widespread use as the prototypical mGluR5 antagonist.

Experiment 1

Experiment 1 examined the antinociceptive effects of the mGluR1 antagonist JNJ16259685 and the mGluR5 antagonist MPEP alone and in combination with morphine in two rat models of acute pain. The warm-water tail withdrawal assay and the hot plate assay were used to assess the antinociceptive effects of these drugs and their selected combinations. Both of these tests have been used successfully in our laboratory (Fischer et al., 2008b) as well as in other studies (Lomas et al., 2008) of opioid analgesia in combination with glutamatergic antagonists.

The warm-water tail withdrawal assay is a model of acute thermal pain in which an animal's tail is placed in a heated water bath, and the latency to withdrawal the tail is measured. The tail withdrawal response is a reflexive pain

behavior. This reflexive test provides insight into the antinociceptive effects of drugs at a spinal level.

The hot plate assay is a model of acute thermal pain in which an animal is placed on a hot plate analgesia meter, and the latency to perform a number of different escape behaviors is measured. Traditionally, these behaviors include fluttering of the hind paw, licking of the hind paw, and jumping to escape the hot plate. All of these behaviors require a locomotor component, thus, this is a test of a more integrated antinociceptive response, as compared to the tail withdrawal test. While this does provide insight into the more integrated antinociceptive effects of drugs, it is important to remember that this is a pain assay which is subject to a drug's locomotor-impairing effects.

The mGluR1 antagonist JNJ16259685 and the mGluR5 antagonist MPEP were tested both alone and in combination with doses of morphine in both of these acute pain assays. The purpose of testing the mGluR antagonists alone was three-fold: 1) to assess any antinociceptive effects that these drugs may have on their own, 2) to increase antagonist doses as high as needed to ensure they were functionally active, and 3) to assess the time course of those functionally active doses.

Experiment 2

Experiment 2 examined the antihyperalgesic effects of the mGluR1 antagonist JNJ16259685 and the mGluR5 antagonist MPEP alone and in combination with morphine in an inflammation-based model of chronic pain. The

capsaicin-induced hyperalgesic tail withdrawal assay was used to study these effects. In this procedure, the inflammatory agent capsaicin, an extract of red peppers, is administered to the distal end of an animal's tail and the latency to withdraw the tail from a previously non-noxious water bath is recorded. One common problem of many chronic pain models is difficulty in controlling for the level of nociception induced by the administered inflammatory agent. Barrett et al. (2002) found that localization of capsaicin-induced inflammation to the tail produced highly consistent levels of nociception under baseline conditions. For this reason, and for the successful use of this assay in previous studies of morphine antihyperalgesia in combination with NMDA antagonism (Lomas et al., 2008), this assay was selected to investigate the effects of μ-opioid/mGluR antagonist combinations in a chronic pain model.

Selected doses of the mGluR1 antagonist JNJ16259685 and the mGluR5 antagonist MPEP were tested both alone and in combination with varying doses of morphine. In testing the mGluR antagonists alone, information about the antihyperalgesic effects of each antagonist when administered alone could be evaluated before combining selected doses with morphine.

CHAPTER 2

METHODS AND MATERIALS

Animals

Adult male Fischer344 rats 2-3 months of age were purchased from Charles River Laboratories (Raleigh, NC). Upon arrival, rats were individually housed in standard Plexiglas cages with *ad libitum* food and water. Rats were habituated to a colony room maintained on a 12-hour light/dark cycle, with lights on at 7:00 AM, for at least 1 week before testing. Rats were handled and exposed to experimenter restraint on at least 3 separate occasions before the beginning of testing. Rats were transported from the colony room to the testing environment each test day and habituated to the test room for at least 1 hour before testing on each occasion. Both the handling and room habituation protocols were followed to minimize the effect of stress and novelty during all test sessions. All experiments were performed in the light phase of the animals' light/dark cycle, between the hours of 10:00 AM and 4:00 PM. No animal was tested more than once per week. All animals used in this study were cared for in accordance with a protocol approved by and the guidelines laid out by the Institutional Animal Care and Use Committee at the University of North Carolina at Chapel Hill.

Drugs

Morphine sulfate and buprenorphine hydrochloride were provided by the National Institute on Drug Abuse (Bethesda, MD). JNJ16259685 and MPEP were purchased from Tocris Biosciences (Ellisville, MO). Isoflurane was purchased from Phoenix Pharmaceuticals (St. Joseph, MO). Capsaicin was purchased from Sigma-Aldrich, Inc. (St. Louis, MO). Morphine sulfate, buprenorphine hydrochloride, and MPEP were dissolved in a 0.9% phosphate-buffered saline solution. JNJ16259685 was dissolved in 45% (w/v) 2-hydroxypropyl-β-cyclodextrin. Capsaicin was dissolved in a solution of Tween 80/95% ethanol/saline in a 1/1/8 ratio, which was further diluted with saline. Morphine sulfate, buprenorphine hydrochloride, and capsaicin were prepared in stock solutions and further diluted with saline for the preparation of selected doses. Selected doses of JNJ16259685 and MPEP were prepared acutely. Morphine, buprenorphine, JNJ16259685 and MPEP were all administered i.p. at a volume of 0.1mL/100g. Capsaicin was administered locally to the tail in a set volume of 0.1 mL. Animals were exposed to isoflurane anesthesia in an inhalational chamber.

Experiment 1

Experiment 1 examined morphine antinociception in combination with selected doses of the mGluR antagonist JNJ16259685 or the mGluR5 antagonist MPEP. Antinociception was assessed using the warm-water tail withdrawal

assay and the hot plate assay. Animals were tested twice in each assay under baseline conditions to yield an average baseline latency measure for each animal. Within each test session, animals were first tested in the tail withdrawal procedure and then in the hot plate procedure. Originally, this test order was counter-balanced, but, pilot investigations indicated baseline response levels were inconsistent when rats were examined on the hot plate prior to the tail withdrawal procedure (data not shown). Therefore, rats were examined first in the tail withdrawal procedure throughout the study. A cumulative morphine dosing procedure was used to obtain each morphine dose-effect curve. The selected mGluR antagonist dose was administered once prior to obtaining each cumulative dose-effect curve. Injections were timed such that the mGluR antagonist was administered 30 min before initiating the morphine dose-effect curve, and each cumulative morphine dose was administered 15 min prior to each test point within the entire session. Maximum latency values were set at 15 sec in the tail withdrawal assay and at 40 sec in the hot plate assay to prevent tissue damage and to allow for the conversion of raw latency data to % antinociceptive effect data. Maximum latency values also differed between tests due to baseline differences in latency values between the two assays, with baseline response latencies averaging 4 sec in the tail withdrawal procedure and 10 sec in the hot plate procedure.

Warm-water Tail Withdrawal Assay

Rats were removed from their home cages and lightly restrained while the distal 7 cm of the tail was placed into a 52℃ water bath. The tail withdrawal latency was recorded. A cutoff limit of 15 sec was used to avoid tissue damage, at which point the animal was removed from testing, and a maximum latency of 15 sec was recorded.

Hot Plate Assay

Immediately following testing in the warm-water tail withdrawal procedure, rats were placed on a hot plate analgesia meter set at 52℃. Latency to lick the hind paw or perform an escape response was recorded. A cutoff limit of 40 sec was used to prevent tissue damage, at which point the rat was removed from the hot plate and returned to the home cage, and a maximum latency of 40 sec was recorded.

Experiment 2

Experiment 2 examined morphine antihyperalgesia in combination with the mGluR1 antagonist JNJ16259685 or the mGluR5 antagonist MPEP. A tail withdrawal procedure, with the introduction of inflammation to the tail, was used to model chronic pain states and assess antihyperalgesia. Due to the administration of the inflammatory agent capsaicin, a cumulative morphine dosing procedure could not be used in this experiment. A maximum latency

value of 15 sec was used in this assay to prevent tissue damage and to allow for the conversion of raw latency data to % antinociceptive effect data.

Capsaicin-induced Hyperalgesic Tail Withdrawal Assay

Prior to the induction of an inflammatory state, each animal was lightly restrained and the distal 7 cm of the tail was immersed in a 45℃ water bath to ensure that each animal's tail would remain immersed at this temperature up to the experimentally imposed maximum latency of 15 sec. Any animal that failed to do so was removed from the experiment. Hyperalgesia was induced by injecting 3.0 µg capsaicin 3.5 cm into the distal end of the tail, under isoflurane anesthesia using an inhalational drop-method. Animals were placed into a chamber prepared with approximately 1.0 mL isoflurane and observed for sedation. Animals were then removed and administered capsaicin locally to the tail and placed back in the home cage. Rats recovered from the procedure within 2-3 min. After administration of capsaicin, tail-withdrawal latencies decreased from 15 sec to an average of 4 sec in response to the same 45℃ water bath.

Before initiating testing, a capsaicin baseline was assessed in each rat. During morphine dose-effect curve test sessions, rats were randomly assigned to receive varying doses of morphine either alone or in selected combinations with either JNJ or MPEP. This administration preceded behavioral testing by 30 min. Capsaicin administration preceded testing by 15 min. During behavioral testing, animals were lightly restrained, the distal 7cm of the tail was immersed, and the latency to withdraw the tail was recorded. For all tests of antihyperalgesia, a

15 sec cutoff latency was used as this indicated a maximal antihyperalgesic effect (i.e., withdrawal latencies returned to pre-inflammation baseline levels).

Selected doses of each mGluR antagonist were also tested alone in this assay. Such tests followed the same time course as described above.

Data Analysis

For all tests of opioid antinociception and antihyperalgesia, raw latency scores were converted to % maximum possible effect scores using the following equation:

$$\% \text{ MPE} = [(\text{observed} - \text{baseline}) / (\text{maximum} - \text{baseline})] \times 100.$$

When possible, the %MPE scores from dose-effect curves examining antinociception and antihyperalgesia were used to mathematically derive the dose of the opioid required to produce a 50% analgesic effect (ED50) either alone or in combination with an mGluR antagonist. Calculation of the ED50 value required the following: 1) an ascending limb of the dose effect curve comprised of at least 3 points and, 2) that the lowest mean %MPE within this limb was 20% or lower, and the highest mean %MPE was 80% or higher.

When the ED50 value could be calculated, relative potency estimates between each opioid given alone and in combination with the mGluR antagonists were also determined. For this analysis, dose ratios were calculated by comparison of the slopes of two linear regression lines representing two dose-effect curves and the distance between those two lines, a method described by Tallarida and Murray (1987). Differences in the relative potency of each opioid

alone and in combination were considered to be significant if the 95% confidence interval did not overlap 1.0 or below.

When calculation of the ED50 value was not possible (i.e., the dose-effect curve did not meet requirements noted above), comparisons were made between the opioid dose-effect curve and the opioid/mGluR antagonist combination dose-effect curves by conducting a two-factor analysis of variance (ANOVA). The opioid and the mGluR antagonist administered were set as the two factors with specific doses of each as the levels of each factor. In post-hoc analyses, the mean analgesic effects of each drug combination were compared to determine specific opioid/mGluR antagonist dose combinations that were significantly different from the opioid administered alone.

For tests of mGluR antagonist functional activity and time course in each antinociceptive and antihyperalgesic test, raw latency scores following drug administration were compared to baseline latency scores by conducting a repeated measures ANOVA with dose as the between-groups factor and time point as the repeated measures factor. The Fisher protected least significant differences (PLSD) test was used in post hoc analyses.

CHAPTER 3

RESULTS

Experiment 1

Experiment 1 examined the antinociceptive effects of the mGluR1 antagonist JNJ16259685 and the mGluR5 antagonist MPEP both alone and in combination with morphine in two models of acute pain: the warm-water tail withdrawal procedure and the hot plate procedure. Figure 1 presents the effects of JNJ on % maximum possible effect in the warm-water tail withdrawal procedure. The bottom portion of Fig 1 (1d – 1f) shows the effects of selected doses of JNJ alone, determined at 15, 30, 45, and 60 min post-injection. Neither 1.0 nor 3.0 mg/kg JNJ significantly increased %MPE at these time points; however, a dose of 5.6 mg/kg JNJ produced an average MPE of approximately 25% at 30, 45, and 60 min post-injection. The top portion of Fig 1 (1a – 1c) presents the effects of JNJ in combination with morphine. The 3.0 mg/kg dose of JNJ and the 5.6 mg/kg dose of JNJ shifted the morphine dose-effect curve leftward, 1.7-fold and 1.9-fold, respectively.

Figure 2 presents the effects of JNJ in the hot plate procedure, with the effects of JNJ alone presented in the bottom portion (2d – 2f). Both the 1.0 mg/kg and the 3.0 mg/kg dose of JNJ increased %MPE in the hot plate

procedure, but these effects were brief and generally only occurred during the first 15-30 min after administration. The 1.0 mg/kg dose of JNJ increased %MPE by 40% at 15 min post injection. At 30 min post-injection, the 3.0 mg/kg dose of JNJ increased %MPE by 22%. The top portion of Fig 2 (2a – 2c) presents the effects of JNJ in combination with morphine in the hot plate procedure. The 1.0 mg/kg dose of JNJ produced a significant 3.5-fold shift to the left in the morphine dose-effect curve. The 3.0 mg/kg dose of JNJ produced a significant 2.6-fold shift to the left in the morphine dose-effect curve.

Figure 3 presents the effects of MPEP on % maximum possible effect in the warm-water tail withdrawal procedure. The bottom portion of Fig 3 (3d – 3f) shows the effects of selected doses of MPEP alone, determined at 15, 30, 45, and 60 min post-injection. Neither 3.0 mg/kg nor 10.0 mg/kg MPEP significantly increased %MPE at these time points; however, a dose of 30.0 mg/kg MPEP produced an MPE of 29% at 30 min post-injection and an MPE of 34% at 45 min post-injection. The top portion of Fig 3 (3a – 3c) presents the effects of MPEP in combination with morphine. The 30.0 mg/kg dose of MPEP shifted the morphine dose-effect curve leftward, 2.3-fold.

Figure 4 presents the effects of MPEP in the hot plate procedure, with the effects of MPEP alone presented in the bottom portion (4d – 4f). Neither the 3.0 mg/kg nor the 10.0 mg/kg dose of MPEP significantly increased %MPE in the hot plate procedure. The 30.0 mg/kg dose of MPEP increased %MPE by 25% at 15 min post-injection and by 21% at 30 min post-injection. The top portion of Fig 4 (4a – 4c) presents the effects of MPEP in combination with morphine in the hot

plate procedure. The 30.0 mg/kg dose of MPEP produced a significant 6.2-fold shift to the left in the morphine dose-effect curve.

Experiment 2

Experiment 2 examined the antihyperalgesic effects of JNJ and MPEP both alone and in combination with morphine in a model of inflammatory pain: the capsaicin-induced hyperalgesic tail withdrawal procedure. Figure 5 presents the effects of JNJ in the capsaicin tail withdrawal procedure. JNJ did not significantly increase %MPE when given alone at any of the doses tested, nor did it produce consistent alterations in the effects of morphine, resulting in no shift in the morphine dose-effect curve.

Figure 6 presents the effects of MPEP in the capsaicin-induced hyperalgesic tail withdrawal procedure. Neither the 1.0 mg/kg dose nor the 3.0 mg/kg dose of MPEP significantly increased %MPE when given alone; however, the 10.0 mg/kg dose of MPEP increased %MPE by 62%. When this dose of MPEP was combined with morphine, it produced a bi-phasic effect on the morphine dose-effect curve, increasing %MPE of low morphine doses and decreasing %MPE of high morphine doses. A two-factor ANOVA revealed a significant main effect of morphine ($F=28.9$, $p<0.001$) and a significant interaction between morphine and MPEP ($F=2.9$, $p<0.05$) but no significant main effect of MPEP. Post hoc analyses revealed that the 10.0 mg/kg dose of MPEP significantly attenuated the effects produced by high doses of morphine and

produced a strong trend ($p<0.1$) toward enhancement of the effects produced by a low dose of morphine.

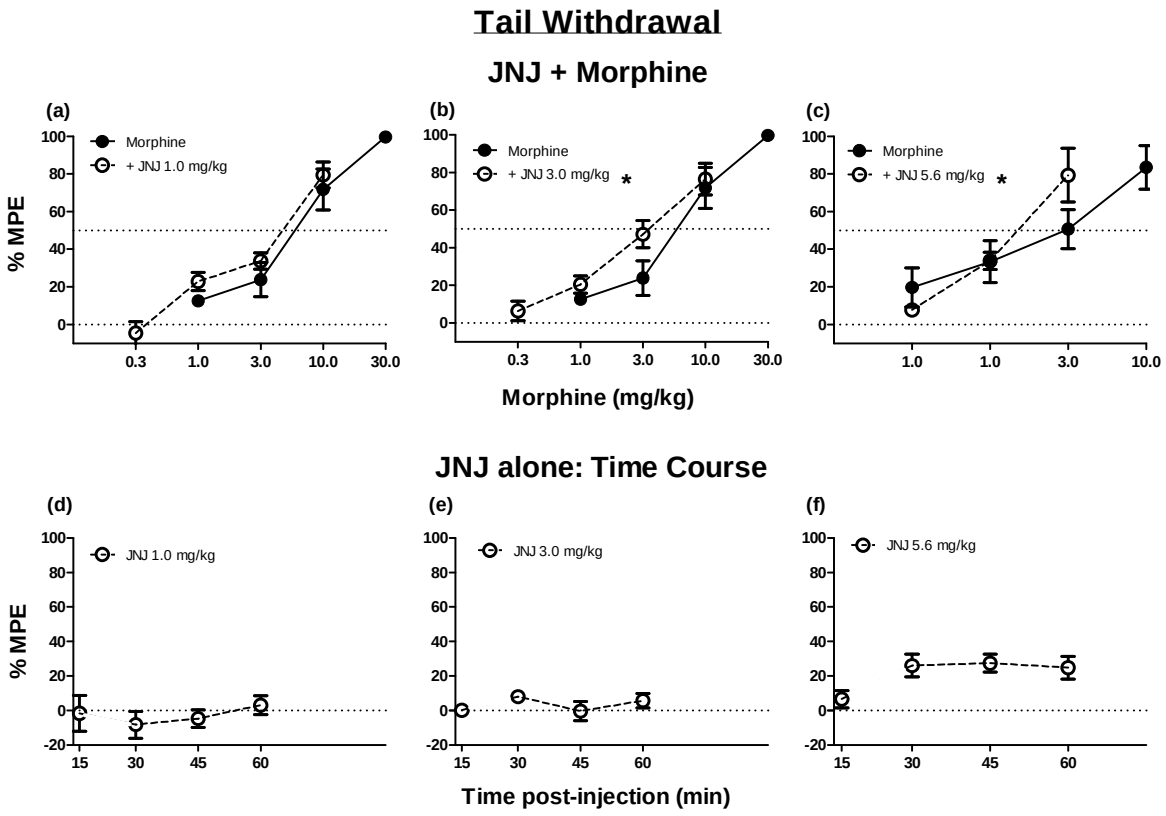


Figure 1. Effects of the mGluR1 antagonist JNJ16259685 on morphine-induced antinociception in the tail withdrawal assay.

Panels 1a-1c: Data are presented as mean %MPE ± S.E.M. on the ordinate as a function of morphine dose on the abscissa. An asterisk (*) represents a significant shift to the left of the morphine dose-effect curve by the given JNJ dose.

Panels 1d-1f: Effects of JNJ when given alone in the tail withdrawal assay. Data are presented as mean %MPE ± S.E.M. on the ordinate as a function of time point following injection on the abscissa. Note that the effects of JNJ in combination with morphine (panels 1a-1c) align vertically between panels with the effects of JNJ when given alone at the same time point post injection (panels 1d-1f).

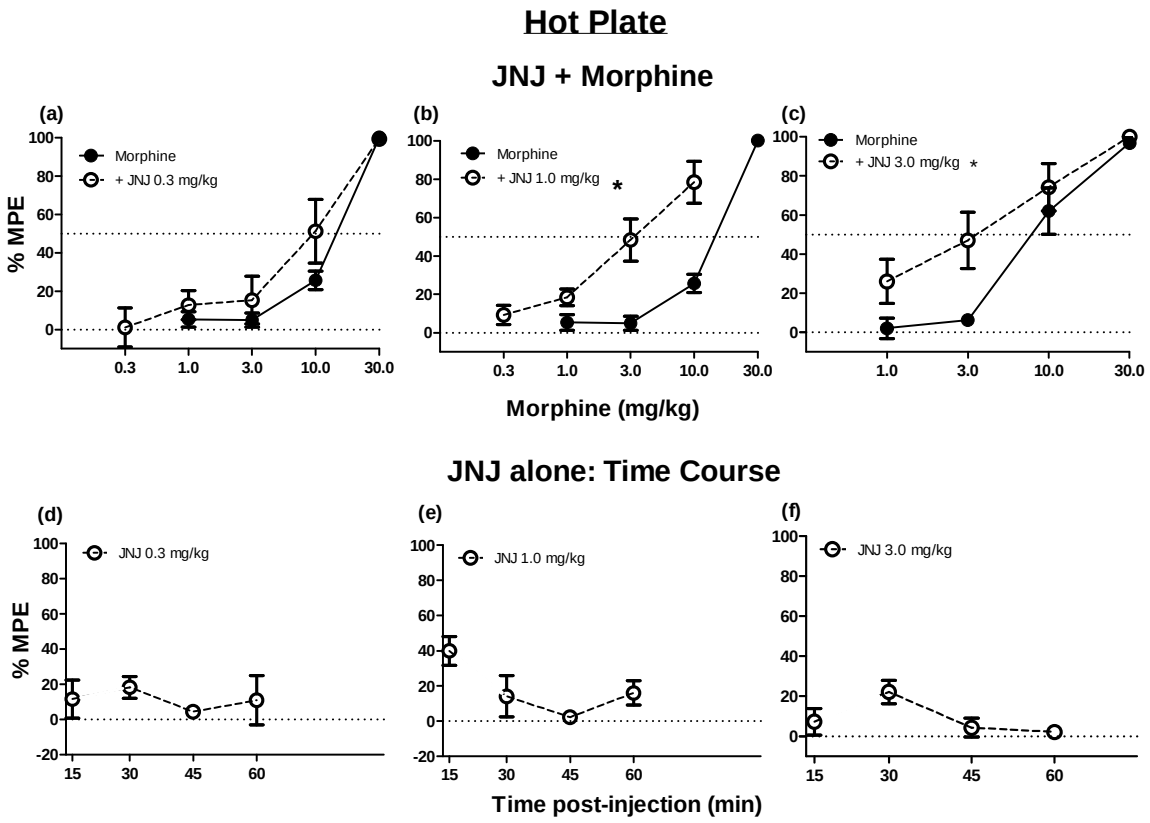


Figure 2. Effects of the mGluR1 antagonist JNJ16259685 on morphine-induced antinociception in the hot plate assay.

Panels 2a-2c: Data are presented as mean %MPE ± S.E.M. on the ordinate as a function of morphine dose on the abscissa. An asterisk (*) represents a significant shift to the left of the morphine dose-effect curve by the given JNJ dose.

Panels 2d-2f: Effects of JNJ when given alone in the hot plate assay. Data are presented as mean %MPE ± S.E.M. on the ordinate as a function of time point following injection on the abscissa. Note that the effects of JNJ in combination with morphine (panels 2a-2c) align vertically between panels with the effects of JNJ when given alone at the same time point post injection (panels 2d-2f).

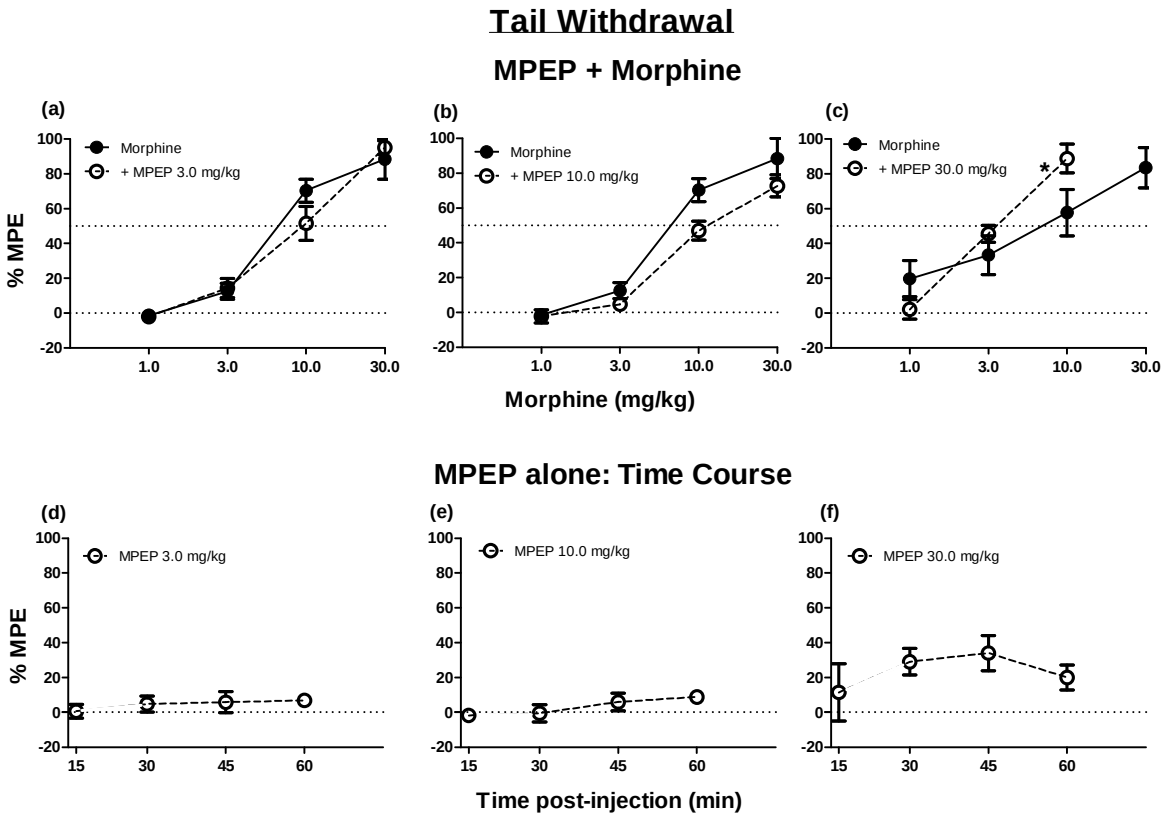


Figure 3. Effects of the mGluR5 antagonist MPEP on morphine-induced antinociception in the tail withdrawal assay.

Panels 3a-3c: Data are presented as mean %MPE ± S.E.M. on the ordinate as a function of morphine dose on the abscissa. An asterisk (*) represents a significant shift to the left of the morphine dose-effect curve by the given MPEP dose.

Panels 3d-3f: Effects of MPEP when given alone in the tail withdrawal assay. Data are presented as mean %MPE ± S.E.M. on the ordinate as a function of time point following injection on the abscissa. Note that the effects of MPEP in combination with morphine (panels 3a-3c) align vertically between panels with the effects of MPEP when given alone at the same time point post injection (panels 3d-3f).

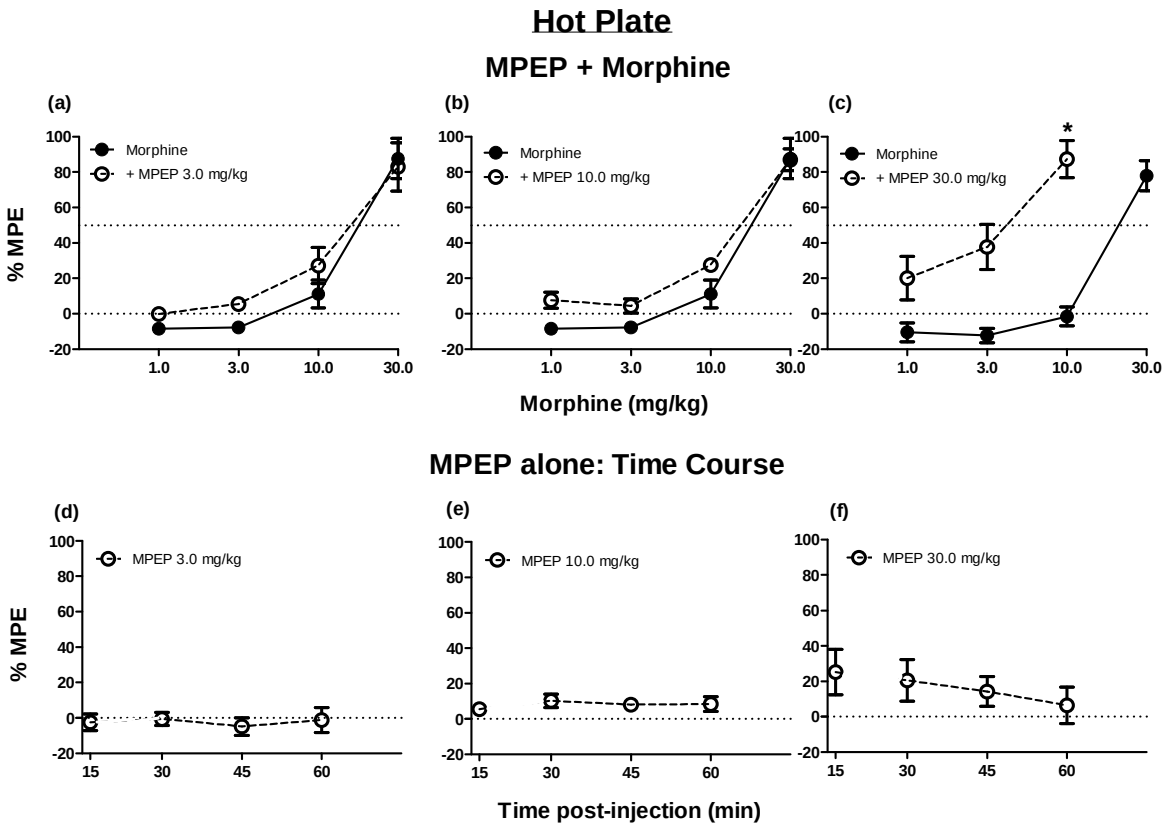


Figure 4. Effects of the mGluR5 antagonist MPEP on morphine-induced antinociception in the hot plate assay.

Panels 4a-4c: Data are presented as mean %MPE ± S.E.M. on the ordinate as a function of morphine dose on the abscissa. An asterisk (*) represents a significant shift to the left of the morphine dose-effect curve by the given MPEP dose.

Panels 4d-4f: Effects of MPEP when given alone in the hot plate assay. Data are presented as mean %MPE ± S.E.M. on the ordinate as a function of time point following injection on the abscissa. Note that the effects of MPEP in combination with morphine (panels 4a-4c) align vertically between panels with the effects of MPEP when given alone at the same time point post injection (panels 4d-4f).

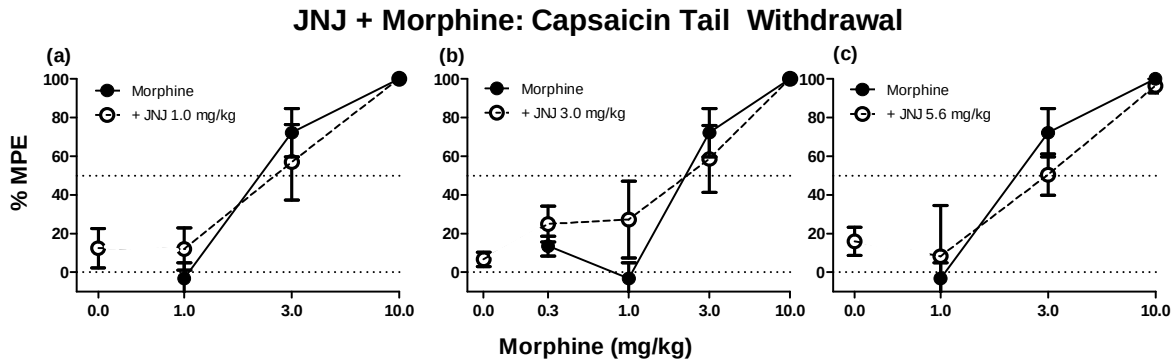


Figure 5. Effects of the mGluR1 antagonist JNJ16259685 on morphine-induced antihyperalgesia in the capsaicin-induced hyperalgesic tail withdrawal assay.

Data are presented as mean %MPE ± S.E.M. on the ordinate as a function of morphine dose on the abscissa. Analysis of ED50 and potency ratio values revealed that no dose of JNJ tested produced a significant shift in the morphine dose-effect curve.

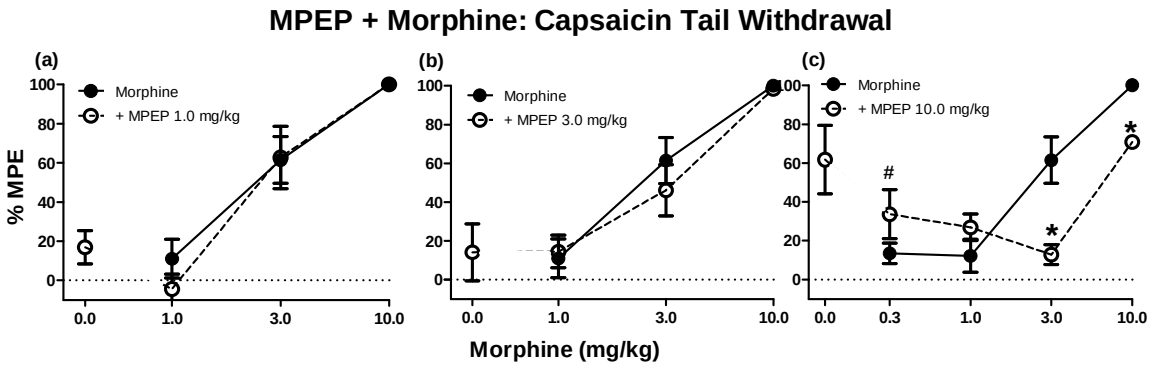


Figure 6. Effects of the mGluR5 antagonist MPEP on morphine-induced antihyperalgesia in the capsaicin-induced hyperalgesic tail withdrawal assay.

Data are presented as mean %MPE ± S.E.M. on the ordinate as a function of morphine dose on the abscissa. An asterisk (*) represents a significant difference ($p<0.05$) between the antihyperalgesic effect produced by a given dose of morphine alone and in combination with MPEP. A pound (#) represents a trend ($p<0.1$) toward a difference in the antihyperalgesic effect produced by a give dose of morphine alone and in combination with MPEP.

CHAPTER 4

DISCUSSION

The present study examined the effects of the mGluR1 antagonist JNJ16259685 and the mGluR5 antagonist MPEP in rat models of acute and inflammatory pain. Experiment 1 examined the antinociceptive properties of JNJ and MPEP in the warm-water tail withdrawal procedure and the hot plate procedure. In both procedures, selective doses of JNJ and MPEP produced antinociception when administered alone and enhanced morphine-induced antinociception.

A 5.6 mg/kg dose of the mGluR1 antagonist JNJ produced significant antinociception when administered alone and shifted the morphine dose-effect curve leftward 1.9-fold in the warm-water tail withdrawal procedure. A slightly lower dose (3.0 mg/kg) did not produce significant antinociception alone in the tail withdrawal procedure but did produce a 1.7-fold shift to the left in the morphine dose-effect curve. In the hot plate procedure, a 3.0 mg/kg dose of JNJ produced significant antinociception when administered alone and produced a significant 2.6-fold shift to the left in the morphine dose-effect curve while a slightly lower dose (1.0 mg/kg) did not produce significant antinociception alone at a relevant time point but did significantly shift the morphine dose-effect curve leftward, 3.5-fold. These data are in agreement with a number of studies

reporting the antinociceptive efficacy of mGluR1 antagonists (Fischer et al., 2001a; Fischer et al., 2001b; Neugbauer et al., 1999).

It is interesting to note that JNJ displayed antinociceptive properties differentially between the two acute pain assays. That is, JNJ produced significant antinociception and significant leftward shifts in the morphine dose-effect curves in both assays, however, the magnitude of the shift was smaller in the tail withdrawal test (1.7 - 1.9-fold) than in the hot plate test (2.6 - 3.2-fold). Furthermore, these results were produced by different doses of JNJ; the 3.0 mg/kg and 5.6 mg/kg doses significantly enhanced morphine antinociception in the tail withdrawal test while the 1.0 mg/kg and 3.0 mg/kg doses did so in the hot plate test. These differences are not entirely surprising as these two tests are differential in nature. The tail withdrawal test requires a reflexive response while the hot plate test requires a more integrated pain response. The differences in the results of JNJ between these two procedures point to the importance of the pain assay used when evaluating the analgesic efficacy of a compound.

Experiment 1 also showed that a 30.0 mg/kg dose of the mGluR5 antagonist MPEP produced significant antinociceptive effects alone. Moreover, this dose of MPEP significantly shifted the morphine-dose effect curve to the left. This effect contrasts with the findings of Fischer et al. (2008a; 2008b) which indicated that MPEP did not enhance μ-opioid-induced antinociception in the tail-flick and hot plate assays. However, there are important differences between the present results and those reported by Fischer et al. First of all, the present study used a rat model while the Fischer et al. studies used mice. Furthermore, the

present study used the warm-water tail withdrawal procedure while the former Fischer et al. (2008a) study used the tail-flick procedure.

It is also important to note that a 30.0 mg/kg i.p. dose of MPEP is a considered to be a relatively high dose which may not be fully selective to mGluR5; Montana et al. (2009) reported that the same dose of MPEP also given i.p. in mGluR5 knockout mice produced significant analgesia in the formalin test, suggesting that MPEP is probably active at another site at this dose, at least in mGluR5 knockout mice. Though MPEP is the prototypical mGluR5 antagonist, a number of studies have cited its inferiority in selectivity for mGluR5 as compared to newer antagonists such as 3-[(2-methyl-1,3-thiazol-4-yl)ethynyl]pyridine (MTEP) and [N-(3-chlorophenyl)-N'-(4,5-dihydro-1-methyl-4-oxo-1H-imidazole-2-yl)urea] (fenobam) (Lea & Faden, 2006; Montana et al., 2009). Therefore, although a 30.0 mg/kg dose of MPEP produced significant antinociception and enhancement of morphine antinociception in two rat models of acute pain, it is likely that these effects are not mediated through the mGluR5 system.

Experiment 2 examined the antihyperalgesic properties of JNJ16259685 and MPEP in a rat model of inflammatory pain: the capsaicin-induced hyperalgesic tail withdrawal procedure. In this experiment, JNJ neither produced antihyperalgesic effects when administered alone nor enhanced morphine-induced antihyperalgesia. These findings are in agreement with a number of other studies which have shown that mGluR1 antagonists are not active in inflammatory pain models (Gabra et al., 2008; Lee et al., 2006; Walker et al., 2001a).

In contrast, a 10.0 mg/kg dose of the mGluR5 antagonist MPEP produced significant antihyperalgesia when given alone in the capsaicin-induced hyperalgesic tail withdrawal procedure. This effect is in agreement with other studies in which selected mGluR5 antagonists, when administered alone, produced antihyperalgesic effects in inflammatory models (Lee et al., 2006; Walker et al., 2001a; Walker et al., 2001b). Interestingly, the 10.0 mg/kg dose of MPEP did not enhance morphine-induced antihyperalgesia. Instead, it appeared to attenuate the analgesic effects of high doses of morphine. Though this result was unexpected, it is not completely unprecedented. Popik et al. (2000) reported a trend toward attenuation in morphine antinociception in the tail flick assay in mice by memantine and MRZ 2/579, antagonists of the NMDA ionotropic glutamate recepotor. Furthermore, Nemmani et al. (2004) reported that another NMDA antagonist, dextromethorphan, enhanced low-dose morphine antinociception but attenuated high-dose morphine antinociception in the warm-water tail withdrawal procedure in mice. Furthermore, the bi-phasic pattern reported in the Nemmani et al. study was also observed in the present study with the 10.0 mg/kg dose of MPEP producing a slight enhancement of the antihyperalgesic effects of low doses of morphine. This enhancement may be difficult to interpret, however, given that 10.0 mg/kg MPEP produced antihyperalgesic effects when administered alone.

Interpreting the results of combinations of morphine and MPEP in the capsaicin procedure may prove difficult. Given the bi-phasic pattern of MPEP's effects on morphine antihyperalgesia, an interaction between the mGluR5 and

MOR receptor systems seems likely. MPEP has been shown to decrease MOR phosphorylation, internalization, and desensitization in HEK293 cells co-expressing mGluR5 and MOR (Schröder et al., 2009). These processes may alter the availability of MOR to its ligands, an effect which may alter the analgesic efficacy of a given dose of a μ-opioid. Receptor occupancy theory states that the pharmacologic response of a drug is proportional to the fraction of the target receptor population occupied by the drug at a given concentration (Ross & Kenakin, 2001). In accordance with this theory, if the number of MORs available to morphine is altered by MPEP, then the number of MORs occupied by morphine is likewise altered, thereby altering the behavioral response. It is possible that under the inflammatory conditions and selected drug doses of the present study, 10.0 mg/kg MPEP may have decreased opiate receptor availability, thereby attenuating the antihyperalgesic effects of high doses of morphine.

Further complicating the interpretation of the combination effects of MPEP and morphine in the capsaicin procedure is the fact that MPEP does display some non-selective activity at other glutamate receptors, most notably mGluR1 and the NMDA receptor (Lea & Faden, 2006). While MPEP and the more recently developed MTEP exhibit equal off-site effect for mGluR1, MPEP displays significantly more off-site activity at the NMDA receptor (Cosford et al., 2003). It is a possibility that 10.0 mg/kg MPEP, under our inflammatory model, may have produced some of its effects through activity at the NMDA receptor. The attenuation of morphine analgesia by this dose of MPEP would then agree

with the attenuation of morphine analgesia seen by other researchers with selected NMDA antagonists in other pain models (Popik et al., 2000; Nemmani et al., 2004). Lastly, post-synaptic mGluR1 and mGluR5 activity has been shown to increase NMDA-mediated responses (Cerne & Randic, 1992; Kelso et al., 1992; Skeberdis et al., 2001), another possible explanation for the unpredicted results of MPEP administration.

The behavioral results of the present study of Group I mGluR antagonists in acute and inflammatory pain modulation are further supported by a number of physiological studies examining the distribution and functionality of mGluR1 and mGluR5 within the nervous system (Dolan et al., 2003; Dolan et al., 2004; Pitcher et al., 2007; Walker et al., 2001b). Functionally, mGluR5 has been shown to be upregulated in response to persistent inflammation (Dolan et al., 2003) and in response to post-surgical pain (Dolan et al., 2004) in sheep. In contrast, mGluR1 expression levels were unaltered under these conditions. Walker et al. (2001b) found that mGluR5 receptors expressed on peripheral nociceptors are important mediators of inflammatory hyperalgesia. Furthermore, Pitcher et al. (2007) examined the intracellular expression patterns of mGluR1 and mGluR5 in response to different pain states and found that only after induction of inflammation, was the mGluR5 receptor trafficked to the plasma membrane, where it is more readily involved in neurotransmission. All the above studies provide demonstrations of inflammation altering mGluR5 levels but not those of mGluR1. These findings provide support for the results of the present study in which the mGluR1 antagonist JNJ16259685 was not implicated in the

inflammatory capsaicin procedure. Furthermore, these findings support the antihyperalgesia produced by a 10.0 mg/kg dose of the mGluR5 antagonist MPEP in the capsaicin procedure.

Future studies examining the effects of Group I mGluR compounds on μ-opioid analgesia might consider a number of other factors. For example, sex is an important determinant in the efficacy of NMDA antagonists to alter μ-opioid analgesia (Lomas et al., 2008; Nemmani et al., 2004). It is not yet clear if this trend also occurs with Group I mGluR anatagonism. Expanding the number of inflammatory/chronic pain models would also be of interest. There are indications that capsaicin administration produces both thermal and mechanical primary hyperalgesia, sensitization of peripheral nociceptive receptors and fibers; however there are also indications that capsaicin administration produces only mechanical secondary hyperalgesia, sensitization of the nociceptive neurons of the central nervous system (Willis, 2009). The present study examined only thermal pain in response to capsaicin administration, meaning it is possible only primary hyperalgesia was examined. It would be interesting to also examine either mechanical hyperalgesia in response to capsaicin or another more integrated inflammatory model such as Complete Freund's Adjuvant to further examine the role of the mGluR system in secondary hyperalgesia, or central sensitization. Additionally, the side effect profile (a large impediment in the clinical utility of NMDA antagonist treatments for pain) of Group I mGluR compounds has not been fully characterized. While some reports indicate that newer mGluR compounds have a more limited side effect profile (Montana et al.,

2009), impairments by mGluR5 antagonists in locomotor activity, rotarod performance and decreases in body temperature have been reported, as well as impairments by both mGluR5 antagonists and mGluR1 antagonists in operant responding for food (Varty et al., 2005).

While the current study examined only Group I mGluR compounds, the prospect of manipulating the Group II mGluR receptors is increasingly promising in the treatment of pain. The Group II family includes the mGluR2 and mGluR3 pre-synaptic autoreceptors. These receptors maintain optimal levels of glutamate neurotransmission through negative feedback mechanisms (Shigemoto & Mizuno, 2000; Cartmell & Schoepp, 2000). Agonists of these receptors, therefore, produce decreases in glutamate activity similar to that of mGluR1 and mGluR 5 antagonists but without direct antagonism.

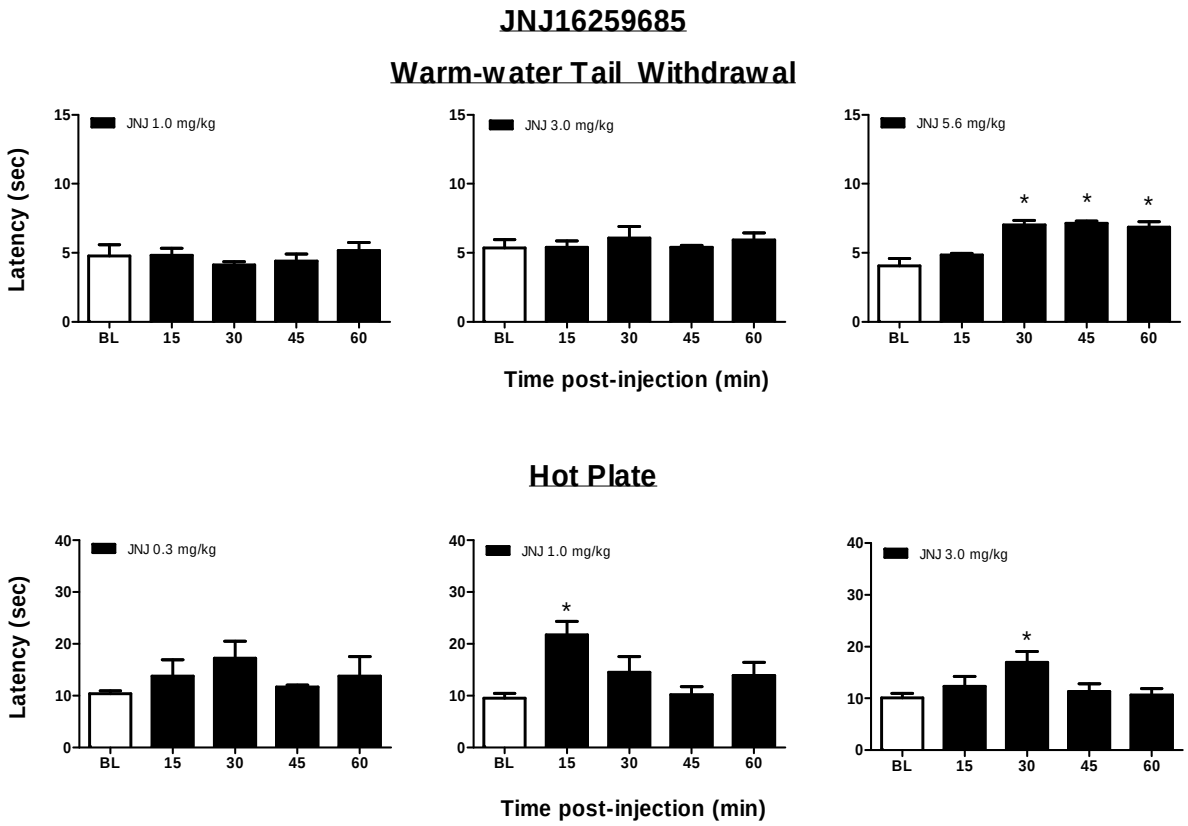
A great number of studies examining mGluR2 and mGluR3 agonists in different pain models indicate that the use of mGluR2/3 agonists may be promising in the management of chronic and inflammatory pain. The results of mGlu2/3 activation in preclinical acute pain models are mixed. For example, Varney & Gereau (2002) point to some involvement of Group II receptors in acute pain while Simmons et al., (2002) found that mGluR2/3 agonists were only efficacious in chronic pain models, not acute pain models. However, the literature is remarkably consistent in the evaluation of mGluR2 and mGluR3 activation in inflammatory and persistent pain states. Group II mGluR activation has been shown to decrease peripheral sensitization in inflammatory states (Du et al., 2008), display analgesic efficacy in response to both acute and repeated

dosing in both persistent and inflammatory pain models (Jones et al., 2005), and to regulate inflammatory receptor function (i.e., the TRPV1 receptor) (Carlton et al., 2009). In addition Dolan et al. found that mGluR3 expression is upregulated following persistent inflammation (2003) and post-surgical pain (2004).

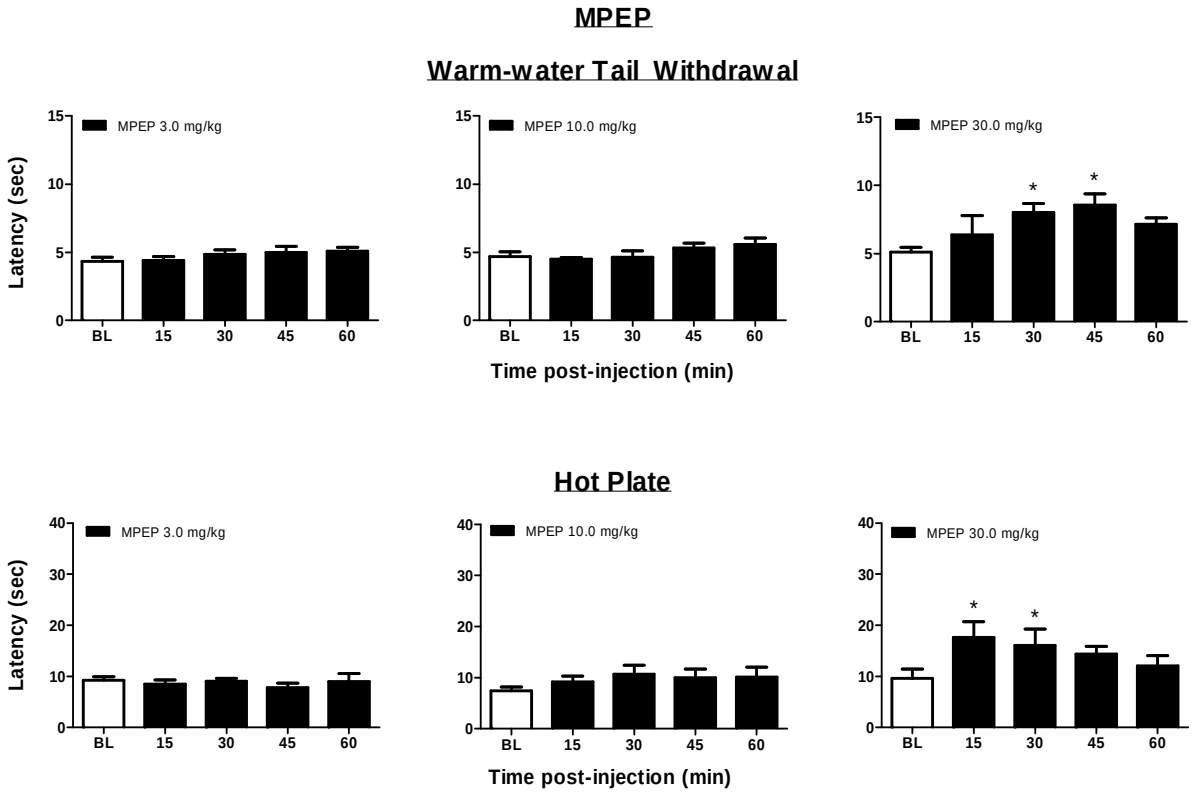
There is much less information concerning the combination of mGluR2/3 compounds with opioid analgesics in the literature. Popik et al. (2000) reported that mGlu2/3 agonists did attenuate the development of analgesic tolerance to morphine, but little has been done to examine the acute effects of mGluR2/3 activation on morphine antinociception or antihyperalgesia. This presents an interesting opportunity to extend the methodology of the present work to mGluR2 and mGluR3 agonists in order to review such effects.

The present study compliments and contributes to a body of literature discriminating between the roles of mGluR1 and mGluR5 in the modulation of pain and μ-opioid-induced analgesia between different pain states. The mGluR1 antagonist JNJ16259685 dose-dependently enhanced morphine-induced antinociception in both the warm-water tail withdrawal and hot plate procedures, but did not alter morphine's effects in the capsaicin-induced hyperalgesic tail withdrawal procedure. The mGluR5 anatonist MPEP did not alter morphine's effects in the warm-water tail withdrawal procedure nor in the hot plate procedure, except at a very high dose which is likely not selective for mGluR5 systems. A slightly lower dose of MPEP produced significant antihyperalgesic effects in the capsaicin-induced hyperalgesic tail withdrawal procedure, but the same dose attenuated high-dose morphine antihyperalgesia. Again, it is possible

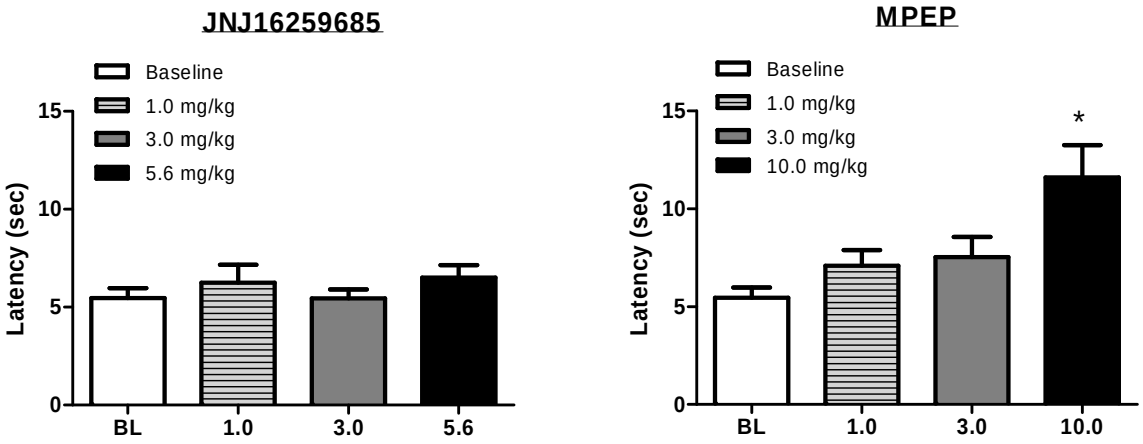
that these results were not related solely to mGluR5 mechanisms. The present data are in line with the hypothesis that the mGluR1 receptor is an important modulator of acute pain, but these data provide limited support for the suggestions that the mGluR5 receptor is an important modulator of inflammatory pain.



Appendix A. Effects of the mGluR1 antagonist JNJ16259685 in the warm-water tail withdrawal and hot plate assays. Data are presented as mean latency ± S.E.M. on the ordinate as a function of time point following injection on the abscissa. An asterisk (*) represents a significant increase from baseline.



Appendix B. Effects of the mGluR5 antagonist MPEP in the warm-water tail withdrawal and hot plate assays. Data are presented as mean latency ± S.E.M. on the ordinate as a function of time point following injection on the abscissa. An asterisk (*) represents a significant increase from baseline.



Appendix C. Effects of the mGluR1 antagonist JNJ16259685 and the mGluR5 antagonist MPEP in the capsaicin-induced hyperalgesic tail withdrawal assay. Left panel, JNJ16259685; right panel, MPEP. Data are presented as mean latency ± S.E.M. on the ordinate as a function of the dose of the antagonist administered on the abscissa. An asterisk (*) represents a significant increase from baseline.

REFERENCES

Allen, R.M. & Dykstra, L.A. (2000). Role of morphine maintenance dose in the development of tolerance and its attenuation by an NMDA receptor antagonist. *Psychopharmacology, 148*, 59-65.

Allen, R.M. & Dykstra, L.A. (2001). N-methyl-D-aspartate receptor antagonists potentiate the antinociceptive effects of morphine in squirrel monkeys. *The Journal of Pharmacology and Experimental Therapeutics, 298*(1), 288-297.

Alvarez, F.J., Villalba R.M., Carr, P.A., Grandes, P., & Somohano, P.M. (2000). Differential distribution of metabotropic glutamate receptors 1a, 1b, and 5 in the rat spinal cord. *The Journal of Comparative Neurology, 422*, 464-487.

Barrett, A.C., Smith, E.S., & Picker, M.J. (2003). Capsaicin-induced hyperalgesia and mu-opioid-induced antihyperalgesia in male and female Fischer344 rats. *The Journal of Pharmacology and Experimental Therapeutics, 307*(1), 237-245.

Belluzzi, J.D., Grant, N., Garsky, V., Sarantakes, D., Wise, C.D., & Stein, L. (1976). Analgesia induced in vivo by central administration of enkephalin in the rat. *Nature, 260*, 625-626.

Bossard, A.E., Guirimand, F., Fletcher, D., Gaude-Joindreau, V., Chauvin, M., & Bouhassira, D. (2002). Interaction of a combination of morphine and ketamine on the nociceptive flexion reflex in human volunteers. *Pain, 98*, 47-57.

Calignano, A., Moncada, S., & Di Rosa, M. (1991). Endogenous nitric oxide modulates morphine-induced constipation. *Biochemistry and Biophysical Research Communications, 181*(2), 889-893.

Carlton, S.M., Du, J., & Zhou, S. (2009). Group II metabotropic glutamate receptor activation on peripheral nociceptors modulates TRPV1 function. *Brain Research, 1248*, 86-95.

Cartmell, J. & Schoepp, D.D. (2000). Regulation of neurotransmitter release by metabotropic glutamate receptors. *Journal of Neurochemistry, 75*, 889-907.

Cerne, R. & Randic, M. (1992). Modulation of AMPA and NMDA responses in rat spinal dorsal horn neurons by trans-1-aminocyclopentane-1,3-dicarboxylic acid. *Neuroscience Letters, 144*, 180-184.

Chen, S.L., Huang, E.Y., Chow, L.H., & Tao, P.L. (2005). Dextromethorphan differentially effects opioid antinociception in rats. *British Journal of Pharmacology*, *144*, 400-404.

Cosford, N.D., Tehrani, L., Roppe, J. et al. (2003). 3-[(2-Methyl-1,3-thiazol-4-yl)ethynyl]pyridine: A potent and highly selective metabotropic glutamate receptor subtype 5 antagonist with anxiolytic activity. *Journal of Medicinal Chemistry, 46*, 204-206.

Dolan, S., Kelly, J.G., Monteiro, A.M., & Nolan, A.M. (2003). Up-regulation of metabotropic glutamate receptor subtypes 3 and 5 in spinal cord in a clinical model of persistent inflammation and hyperalgesia. *Pain, 106*(3), 501-512.

Dolan, S., Kelly, J.G., Monterio, A.M., & Nolan, A.M. (2004). Differential expression of central metabotropic glutamate receptor (mGluR) subtypes in a clinical model of post-surgical pain. *Pain, 110*, 369-377.

Du, J., Zhou, S., & Carlton, S.M. (2008). Group II metabotropic glutamate receptor activation attenuates peripheral sensitization in inflammatory states. *Neuroscience, 154*(2), 754-766.

Henry, F.E., Lomas, L., Picker, M.J., & Dykstra, L.A. (2008, June). The NMDA receptor antagonists LY235959 and dextromethorphan enhance morphine's effectiveness in a test of capsaicin-induced hyperalgesia. Poster session presented at the annual meeting of the College on Problems of Drug Dependence, San Juan, Puerto Rico.

Hughes, J., Smith, T., Kosterlitz, H., Fothergill, L., Morgan, B., & Morris, H. (1975). Identification of two related pentapeptides from the brain with potent opiate agonist activity. *Nature, 258*, 577-580.

Fischer, B.D., Carrigan, K.A., & Dykstra, L.A. (2005). Effects of *N*-methyl-D-aspartate receptor antagonists on acute morphine-induced and l-methadone-induced antinociception in mice. *The Journal of Pain, 6*(7), 425-433.

Fischer, B.D. & Dykstra, L.A. (2006). Interactions between an NMDA antagonist and low-efficacy opioid receptor agonists in assays of schedule-controlled responding and thermal nociception. *The Journal of Pharmacology and Experimental Therapeutics, 318*(3), 1300-1306.

Fischer, B.D., Zimmerman, E.I., Picker, M.J., & Dykstra, L.A. (2008a). Morphine in combination with metabotropic glutamate receptor antagonists on schedule-controlled responding and thermal nociception. *The Journal of Pharmacology and Experimental Therapeutics, 324*(2), 732-739.

Fischer, B.D., Miller, L.L., Henry, F.E., Picker, M.J., & Dykstra, L.A. (2008b). Increased efficacy of μ-opioid agonist-induced antinociception by metabotropic glutamate receptor antagonists: Comparison with (-)-6-phosphonomethyl-deca-hydroisoquinoline-3-carboxylic acid (LY235959). *Psychopharmacology, 198*(2), 271-278.

Fisher, K. & Coderre, T.J. (1996). The contribution of metabotropic glutamate receptors (mGluRs) to formalin-induced nociception. *Pain, 68*, 255-263.

Fundytus, M.E., Yashpal, K., Chabot, J.G., Osborne, M.G., Lefebvre, C.D., Dray, A., Henry, J.L., & Coderre, T.J. (2001). Knockdown of spinal metabotropic glutamate receptor 1 (mGluR(1)) alleviates pain and restores opioid efficacy after nerve injury in rats. *British Journal of Pharmacology, 132*(1), 354-367.

Gabra, B.H., Smith, F.L., Navarro, F.L., Carroll, F.I., & Dewey, W.L. (2008). mGluR5 antagonists that block calcium mobilization in vitro also reverse (S)-3,5-DHPG-induced hyperalgesia and morphine antinociceptive tolerance in vivo. *Brain Research*, *1187*, 58-66.

Galer, B.S., Lee, D., Ma, T., Nagle, B., & Schlagheck, T.G. (2005). MorphiDex (morphine sulfate/dextromethorphan hydrobromide combination) in the treatment of chronic pain: three multicenter, randomized, double-blind, controlled clinical trials fail to demonstrate enhanced opioid analgesia or reduction in tolerance. *Pain, 115*, 284-295.

Gasparini, F., Lingenhohl, K., Stoehr, N., Flor, P.J., Heinrich, M., Vranesic, I., Biollaz, M., Allegier, H., Heckendorn, R., Urwyler, S., et al. (1999). 2-Methyl-6(phenylethynyl)-pryidine (MPEP), a potent, selective and systemically active mGlu5 receptor antagonist. *Neuropharmacology, 38*, 1493-1503.

Jia, H., Rustioni, A., & Valtschanoff, J.G. (1999). Metabotropic glutamate receptors in superficial laminae of the rat dorsal horn. *The Journal of Comparative Neurology, 410*, 627-642.

Jones, C.K., Eberle, E.L., Peters, S.C., Monn, J.A., & Shannon, H.E. (2005). Analgesic effects of the selective group II (mGlu2/3) metabotropic glutamate receptor agonists LY379268 and LY389795 in persistent and inflammatory pain models after acute and repeated dosing. *Neuropharmacology, 49*, 206-218.

Kelso, S.R., Nelson, T.E., & Leonard, J.P. (1992). Protein kinase C-mediated enhancement of NMDA currents by metabotropic glutamate receptors in *Xenopus oocytes*. *Journal of Physiology, 449*, 705-718.

Koch, T. & Höllt, V. (2008). Role of receptor internalization in opioid tolerance and dependence. *Pharmacology and Therapeutics, 117*(2), 199-206.

Kozela, E., Pilc, A., & Popik, P. (2003). Inhibitory effects of MPEP, an mGluR5 antagonist, and memantine, and *N*-methyl-D-aspartate receptor antagonist, on morphine antinociceptive tolerance in mice. *Psychopharmacology, 165*(3), 245-251.

Krarup, C. (2003). An update on electrophysiological studies in neuropathy. *Current Opinions in Neurology, 16*, 603-612.

Kumar, N., Laferriere, A., Yu, J.S., Poon, T., & Coderre, T.J. (2010). Metabotropic glutamate receptors (mGluRs) regulate noxious stimulus-induced glutamate release in spinal cord dorsal horn of rats with neuropathic and inflammatory pain. *Journal of Neurochemistry, 114*(1), 281-290.

Lavreysen, H., Wouters, R., Bischoff, F., Nobrega Pereira, S., Langlois, X., Blokland, S., Somers, M., Dillen, L., & Lesage, A.S. (2004). JNJ16259685, a highly potent, selective and systemically active mGlu1 receptor antagonist. *Neuropharmacology, 47*, 961-972.

Lea IV, P.M. & Faden, A.I. (2006). Metabotropic glutamate receptor subtype 5 antagonists MPEP and MTEP. *CNS Drug Reviews, 12*(2), 149-166.

Lee, H.J., Choi, H.S., Ju, J.S., Bae, Y.C., Kim, S.K., Yoon, Y.W., & Ahn, D.K. (2006). Peripheral mGluR5 antagonist attenuated craniofacial muscle pain and inflammation but not mGluR1 antagonist in highly anesthetized rats. *Brain Research Bulletin, 70*, 378-385.

Lomas, L.M., Terner, J.M., & Picker, M.J. (2008). Sex differences in NMDA antagonist enhancement of morphine-antihyperalgesia in a capsaicin model of persistent pain: Comparisons to two models of acute pain. *Pharmacology, Biochemistry, and Behavior, 89*(2), 127-136.

Montana, M.C., Cavallone, L.F., Stubbert, K.K., Stefanescu, A.D., Kharasch, E.D., & Gereau IV, R.W. (2009). The metabotropic glutamate receptor subtype 5 antagonist fenobam is analgesic and has improved in vivo selectivity compared with the prototypical antagonist 2-methyl-6-(phenylethynyl)-pyridine. *The Journal of Pharmacology and Experimental Therapeutics, 330*(3), 834-843.

Nemmani, K.V.S., Grisel, J.E., Stowe, J.R., Smith-Carliss, R., & Mogil, J.S. (2004) Modulation of morphine analgesia by site-specific N-methyl-D-aspartate receptor antagonists: Dependence on sex, site of antagonism, morphine dose, and time. *Pain, 109*, 274-283.

Neugbauer, V., Chen, P.S., & Willis, W.D. (1999). Role of metabotropic glutamate receptor subtype mGluR1 in brief nociception and central sensitization of primate STT cells. *The Journal of Neurophysiology, 82*(1), 272-282.

Nishimura, S.L., Recht, L.D., & Pasternak, G.W. (1984). Biochemical characterization of high affinity ^{3}H-opiate binding: further evidence for mu_1 sites. *Molecular Pharmacology, 25*, 29-37.

Pasternak, G.W. (1981). Opiate, enkephalin, and endorphin analgesia: relations to a single population of opiate receptors. *Neurology, 31*, 1311-1315.

Pitcher, M.H., Ribeiro-da-silva, A., & Coderre, T.J. (2007). Effects of inflammation on the ultrastructural localization of spinal cord dorsal horn Group I metabotropic glutamate receptors. *The Journal of Comparative Neurology, 505*, 412-423.

Popik, P., Kozela, E., & Pilc, A. (2000). Selective agonists of group II glutamate metabotropic receptors, LY354740, inhibits tolerance to the analgesic effects of morphine in mice. *British Journal of Pharmacology, 130*, 1425-1431.

Redwine, K.E. & Trujillo, K.A. (2003). Effects of NMDA receptor antagonists on acute μ-opioid analgesia in the rat. *Pharmacology, Biochemistry, and Behavior, 76*(2), 361-372.

Ross, E.M. & Kenakin, T.P. (2001). Pharmacodynamics: mechanisms of drug action and the relationship between drug concentration and effect. In J.G. Hardman, & L.E. Limbird (Eds.), *Goodman and Gilman's the Pharmacological Basis of Therapeutics* (10th ed.) (pp. 31-43). McGraw Hill.

Schröder, H., Wu, D.F., Seifert, A., Rankovic, M., Schulz, S., Höllt, V., & Koch, T. (2009). Allosteric modulation of metabotropic glutamate receptor 5 affects phosphorylation, internalization, and desensitization of the μ-opioid receptor. *Neuropharmacology, 56*(4), 768-778.

Shigemoto, R., & Mizuno, N. (2000). Metabotropic glutamate receptors - immunocytochemical and in situ hybridisation analysis. In O.P. Ottersen OP & J. Storm-Mathisen (Eds.), *Handbook of Chemical Neuroanatomy* (18th Ed.) (pp. 63-98). Amsterdam: Elsevier.

Simmons, R.M.A., Webster, A.A., Kalra, A.B., & Iyengar, S. (2002). Group II mGluR receptor agonists are effective in persistent and neuropathic pain models in rats. *Pharmacology, Biochemistry, and Behavior, 73*, 419-427.

Skeberdis, V.A., Lan, J., Opitz, T., Zheng, X., Bennett, M.V., & Zukin, R.S. (2001). mGluR1-mediated potentiation of NMDA- receptors in involves a rise in intracellular calcium and activation of protein kinase C. *Neuropharmacology, 40*, 856-865.

Smith, F.L., Smith, P.A., Dewey, W.L., & Javed, R.R. (2004). Effects of mGlu1 and mGlu5 metabotropic glutamate antagonists to reverse morphine tolerance in mice. *European Journal of Pharmacology, 492*, 137-142.

Tallarida, R.J. & Murray, R.B. (1987). *Manual of pharmacologic calculations with computer programs*. Berlin: Springer.

Todd, A.J. & Ribeiro da Silva, A. (2005). Molecular architecture of the dorsal horn. In: Hunt, S. & Koltzenburg, M., (Eds.). *The Neurobiology of Pain*. New York: Oxford University Press.

Toll, L. (1995). Intact cell binding and the relation to opioid activities in SH-SY5Y cells. *The Journal of Pharmacology and Experimental Therapeutics, 273*(2), 721-727.

Trescot, A.M., Datta, S., Lee, M., & Hansen, H. (2008). Opioid Pharmacology. *Pain Physician, 11*(2 Supplemental), S133-S153.

Trujillo, K.A. & Akil, H. (1991). Inhibition of morphine tolerance and dependence by the NMDA receptor antagonist MK-801. *Science, 251*, 85-87.

Varney, M.A. & Gereau IV, R.W. (2002). Metabotropic glutamate receptor involvement in models of acute and persistent pain: prospects for the development of novel analgesics. *Current Drug Targets - CNS & Neurological Disorders, 1*, 283-296.

Varty, G.B., Grilli, M., Forlani, A., Fredduzzi, S., Grzelak, M.E., Guthrie, D.H., Hodgson, R.A., Lu, S.X., Nicolussi, E., Pond, A.J., Parker, E.M., Hunter, J.C., Higgins, G.A., Reggiani, A., & Bertorelli, R. (2005). The antinociceptive and anxiolytic-like effects of the metabotropic glutamate receptor 5 (mGluR5) antagonists, MPEP and MTEP, and the mGluR1 antagonist, LY456236, in rodents: a comparison of efficacy and side-effect profiles. *Psychopharmacology, 179*(1), 207-217.

Walker, K., Bowes, M., Pansear, M., Davis, A., Gentry, C., Kesingland, A., Gasparini, F., Spooren, W., Stoehr, N., & Pagano, A. (2001a).

Metabotropic glutamate receptor subtype 5 (mGlu5) and nociceptive function I: Selective blockade of mGlu5 receptors in models of acute, persistent and chronic pain. *Neuropharmacology, 40*(1), 1-9.

Walker, K., Reeve, A., Bowes, M., Winter, J., Witherspoon, G., Davis, A., Schmid, P., Gasparini, F., Kuhn, R., & Urban, L. (2001b). mGlu5 receptors and nociceptive function II: mGlu5 receptors functionally expressed on peripheral sensory neurons mediate inflammatory hyperalgesia. *Neuropharmacology, 40*(1), 10-19.

Willis, W.D. (2009). The role of TRPV1 receptors in pain evoked by noxious thermal and chemical stimuli. *Experimental Brain Research, 196*, 5-11.

Young, M.R., Fleetwood-Walker, S.M., Dickinson, T., Blackburn-Monro, G., Sparrow, H., Birch, P.J., & Bountra, C. (1997). Behavioral and electrophysiological evidence supporting a role for group I metabotropic glutamate receptors in the mediation of nociceptive inputs to the rat spinal cord. *Brain Research, 777*, 161-169.