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Antinociceptive Action of Mitragynine and its Synthetic Analogs in Mice

La Donna Evette Franklin

ANTINOCICEPTIVE ACTION OF MITRAGYNINE AND ITS SYNTHETIC ANALOGS IN MICE.

By
LaDonna Evette Franklin

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Approved by

Advisor: Dr. Abir El-Alfy

Reader: Dr. Marvin Wilson

Reader: Dr. Susan Pedigo

ABSTRACT

Mitragynine is a major alkaloid extracted from the leaves of the Thai traditional herb, *Mitragyna speciosa* Korth (Rubiaceae). The extracts of this plant have been used as substitutes for opium and recent studies have reported that mitragynine possesses antinociceptive actions mediated via the mu opioid receptors. Nonetheless, structural features of mitragynine do not fit the classical opiate ligands. Thus the primary objectives of this study were to evaluate the antinociceptive effect of mitragynine and its synthetic analogs to determine the molecular basis of mitragynine binding to opioid receptors in order to provide better understanding of the potential therapeutic roles of these compounds. The antinociceptive effect of intraperitoneal (i.p.) injection of mitragynine (10-30 mg/kg) was investigated as compared to morphine (5-20 mg/kg) using the tailflick and hotplate tests. In both tests, mitragynine exerted a dose-dependent antinociceptive activity that was maximal at 30 min but was less potent than morphine. Such effects were completely abolished by pretreatment with the opioid receptor antagonist naloxone (10 mg/kg, i.p.). The synthetic mitragynine analogs MC 183, MC186, and MC 187 exhibited no antinociceptive activity at 30 mg/kg dose (i.p.) in either the hotplate or tailflick tests. The present study will provide better insight into the mechanism of action of mitragynine as well as the pharmacology of opioid receptors. Such understanding may lead to the generation of a new class of analgesics of potential therapeutic value.

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I. INTRODUCTION

According to the American Academy of Pain Management, nearly 50 million Americans suffer from chronic pain caused by disease, injury, or disorder (Weiner, 2002). Furthermore, 25 million Americans suffer from acute pain due to surgery or accident (Weiner, 2002). Some experts estimate that approximately $100 billion is spent each year to treat pain (Wallis, 2005).

A. Pain and its Pathways

Pain is the body's defense mechanism against stimuli that will damage its tissues, and it is detected by sensory receptors called nociceptors. Pain triggers a reflexive component that allows the organism to immediately remove itself from the noxious stimuli and an emotional component that encourages the organism to avoid the behavior that resulted in the pain. The three categories of nociceptors are mechanical, thermal, and polymodal nociceptors (Sherwood, 2007). Once stimulated, these receptors transmit signals to the central nervous system via two types of afferent fibers: A-delta fibers and C fibers. The cell bodies of these fibers are located in the dorsal root ganglia and end in the gray matter of the dorsal horn of the spinal cord, terminating on projection neurons that transmit pain signals to higher centers of the brain.

Mechanical and thermal nociceptors transmit signals through A-delta fibers which are large in diameter and myelinated (Meyer, 2005). The large diameter of the fibers enables the organism to recognize the location of the noxious stimuli. The myelinated axons allow action potentials to be conducted very quickly and results in early pain. Early pain travels via the spinothalamic tract from the spinal cord to the posteroventrolateral nucleus, a part of the thalamus, to the primary and then secondary

somatosensory cortex (Sherwood, 2007). The primary somatosensory cortex provides sensory discrimination of pain, and the secondary cortex allows for the detection of pain and memory of previously painful experiences.

Polymodal nociceptors transmit signals through C fibers which are small in diameter and unmyelinated. The unmyelinated, small-diameter axons cause action potentials to be conducted at a significantly slower rate than the action potentials of the A-delta fibers, and this is known as late pain (Sherwood, 2007). Late pain is transmitted via the same pathway as early pain but diverges at the thalamus, yielding additional collaterals to various other brain structures such as the hypothalamus, amygdala, and the anterior cingulated cortex, which plays a key role in pain affect, attention, and motor responses (Sherwood, 2007).

B. Classes of Analgesics

Pain is treated with non-narcotic or narcotic analgesics. The two major classes of the non-narcotic analgesics are paracetamol (acetaminophen) and non-steroidal anti-inflammatory drugs (NSAIDs) (Minneman, 2005). The narcotics consist of another class of drugs known as the opioids, and this class includes natural narcotics, the synthetic narcotics, and the endogenous opioids (Katzung, 1998). Both classes of drugs relieve pain by inhibiting different components of the pain pathway previously described, and those differences account for the effectiveness of the drug's analgesic activity as well as the side effects that result. The non-narcotics are effective in treating minor to moderate pain and are associated with a few side effects. The narcotics are the most effective painkillers known to man and are used to treat moderate to severe pain. However, the

side effects associated with narcotics are extremely serious, ranging from nausea and vomiting to addiction.

C. Non-narcotic Analgesics

Paracetamol, an analgesic and antipyretic, is the active metabolite of phenacetin (Stringer, 2006) and an over-the-counter drug commonly used to reduce fever and relieve various aches and minor pains. Paracetamol can be combined with low doses of other NSAIDs or opioid analgesics to treat more severe pain resulting in a cumulative reduction of side effects (Bertolini, 2006). Paracetamol is the active ingredient of the brand named drugs Excedrin, Goody's, and Tylenol as well as several others (NLM/NIH Online, 2007) and it relieves pain by reducing production of prostaglandins, fatty acid derivatives, that enhance the receptor response to noxious stimuli (Bertolini, 2006). Paracetamol is not as potent as the NSAIDs or opiates, but comparatively, it is safer because it does not irritate the lining of the stomach or affect blood coagulation as much as the NSAIDs. Furthermore, paracetamol does not have high risk for addiction, dependence, tolerance, or withdrawal, which are all side effects of opioids (Bertolini, 2006). However, frequent use of paracetamol may result in liver damage (Stringer, 2006).

Similar to paracetamol, non-steroidal anti-inflammatory drugs (NSAIDs) have analgesic and antipyretic effects. In addition, NSAIDs have anti-inflammatory and antithrombotic effects (Stringer, 2006). Because they vary in potency, duration of action, and elimination from the body, not all NSAIDs are available over-the-counter. They are used to treat inflammation, various types of mild to moderate aches and pains, and fever (NLM/NIH Online, 2007). Aspirin, ibuprofen, and ketorolac are just a few of

the commonly used NSAIDs. Similar to paracetamol, NSAIDs relieve pain by acting on prostaglandin synthesis. However, NSAIDs completely inhibit the production of prostaglandins, including those which protect the lining of the stomach, and NSAIDs also inhibit the production of thromboxanes, which support platelet aggregration. This leads to some of the NSAIDs most serious side effects: stomach ulcers, bleeding, diarrhea, and even kidney and liver failure (Stringer, 2006). NSAIDs are effective in treating inflammation, unlike paracetamol, and they can be used to relieve pain without the side effects of opioids (sedation, respiratory depression, high addiction rate).

D. Narcotic Analgesics

All opiate drugs belong to the last major class of drugs to be discussed, the narcotics. Opiates have morphine-like action in the body whether they are synthetic or occur naturally. As a class, they are currently the most effective painkillers, and in addition to relieving pain, opiates produce a sense of well-being and euphoria (Meyer, 2005). These drugs have various side effects and at high doses, opiates can be lethal.

Opiates have been widely used for medicinal and recreational purposes for centuries. The source of the opiates is opium, an extract of the poppy plant which successfully grows in England and Denmark (Meyer, 2005). However, the opium poppy is primarily cultivated in Southeast Asia, India, China, Iran, Turkey, and southeastern Europe. Opium is prepared by collecting and drying the milky substance that pours from the sliced seed capsules of the poppy just before the plant ripens (Meyer, 2005).

Opium's active ingredient is morphine (Fig. 1A), but other constituents include, thebaine and codeine (Katzung, 1998). Due to their effectiveness in treating pain, pharmacologists have spent years modifying and synthesizing the active ingredients of

opium in an attempt to minimize side effects, produce variations in potency, duration of action, and oral effectiveness. Several semi-synthetic narcotics have been produced from morphine and thebaine such as heroin and oxycodone, respectively (Katzung, 1998). Other completely synthetic narcotics have been produced such as fentanyl, meperidine, and methadone. Although several forms of the natural narcotics exist, all of them produce the side effects commonly associated with morphine (Meyer, 2005). Scientists realized that since the body contained specific receptors that these drugs bind to, then the body must also have endogenous ligands that bind to these receptors as well. This idea led to the discovery of the endogenous opioids and their relationship with opiate receptor subtypes (Meyer, 2005).

Endorphins, dynorphins, and enkephalins are the natural ligands that were discovered that bind to opiate receptors (Minneman, 2005). Once in the brain, natural narcotics and synthetic narcotics mimic these endogenous opioids by binding to opiate receptors. Three important subtypes of opiate receptors have been characterized so far: μ (mu), δ (delta), and κ (kappa) (Katzung, 1998).

The μ-receptors have the highest affinity for the opiates. They are present throughout the brain and spinal cord (Meyer, 2005). The areas of the brain abundant in μ-receptors play a role in morphine-induced analgesia (e.g., the medial thalamus, periaqueductal gray, median raphe, and clusters within the spinal cord); positive reinforcement (nucleus accumbens); cardiovascular and respiratory depression; cough control; nausea and vomiting (brain stem); and sensorimotor integration (thalamus, striatum) (Meyer, 2005).

The δ-receptors are primarily found in the same areas of the brain as μ-receptors but are more restricted. They play a role in modulating olfaction, motor integration, reinforcement, and cognitive function (Meyer, 2005). The regions of the brain rich in δ-receptors are the neocortex, striatum, olfactory areas, substantia nigra, and nucleus accumbens. Overlapping of the δ-receptors and μ-receptors suggests an analgesic mechanism mediated both spinally and supraspinally (Meyer, 2005).

The κ-receptors are even more restricted than the δ-receptors. They are found in the striatum, amygdala, hypothalamus, and pituitary. These receptors modulate water balance, temperature regulation, and neuroendocrine behavior and play a role in pain perception, gut motility, and dysphoria (Meyer, 2005).

The endogenous ligands are able to bind to these receptors once the larger peptides are cleaved (Meyer, 2005). Both biologically active and non-active opioid peptides are released upon cleavage of the large peptides which are pro-opiomelanocortin (POMC), prodynorphin, and proenkephalin (Meyer, 2005). The biologically active opioid peptides released are endorphins (mu receptor ligands), dynorphins (kappa receptor ligands), and enkephalins (delta receptor ligands) (Minneman, 2005). These large peptides are cleaved by proteases into smaller peptides that are packaged into vesicles, transported down the axon, and released at the synapse (Meyer, 2005).

Once the smaller peptides are released at the synapse, they bind to their respective receptor (Fig. 1), which is G protein linked (Katzung, 1998). Consequently, K^+ channels open, Ca^{2+} channels close, and adenylyl cyclase activity is inhibited (Minneman, 2005). At the cellular level, this results in a reduction in cell excitability, followed by inhibition of cell firing, which ultimately leads to a decrease in the release of neurotransmitter. The

neurotransmitters that would normally be released in response to pain include norepinephrine, dopamine, and perhaps most importantly substance P and glutamate (Sherwood, 2007).

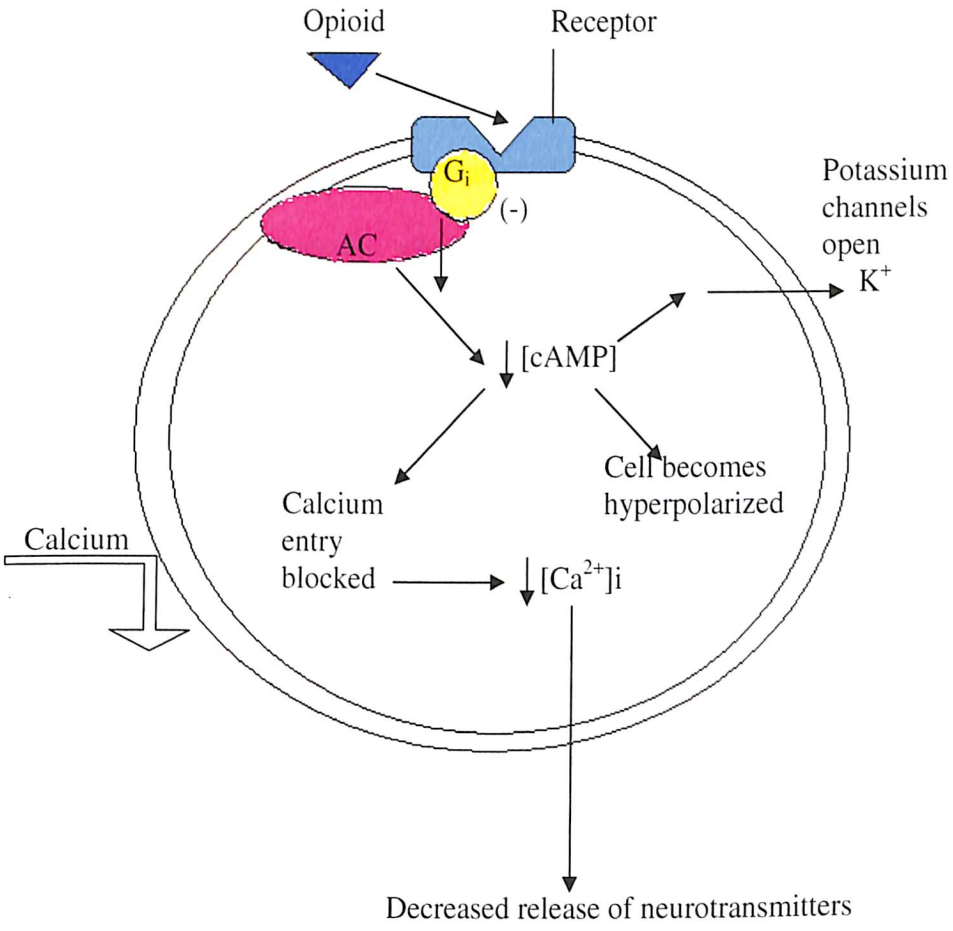


Figure 1 Opioid action at the cellular level. Opioids bind to the G_i protein linked receptor, resulting in a decrease in adenylyl cyclase (AC) activity. This results in a decrease in cyclic adenosine monophosphate (cAMP) production, which increases potassium (K^+) efflux and decreases calcium (Ca^{2+}) influx. Ultimately, this leads to a decrease in the release of neurotransmitter (Minneman, 2005).

Substance P is released from afferent pain fibers and activates ascending pathways that send nociceptive signals to higher brain centers including the cortex (pain localization), thalamus (pain perception), and the reticular formation (alertness). Other areas, such as the hypothalamus and limbic system, which are both important in pain perception, are indirectly affected by substance P (Sherwood, 2007).

Glutamate is released from primary afferent pain fibers and acts on dorsal horn cell. It binds with AMPA receptors, which ultimately leads to the transmission of the pain signals to higher brain centers. Glutamate also binds to NMDA receptors, which leads to Ca^{2+} entry into the dorsal horn cell. As a result, second-messanger systems are activated that make the cell more excitable, and this ultimately leads to a heightened sensitivity to the injured area.

As stated previously, the natural and synthetic analgesics act in a similar way to relieve pain. They reduce the transmission of the pain signals by inhibiting the spinal projection neurons. This prevents pain perception at higher brain centers (supraspinally). Opiates also modulate descending pathways that inhibit spinal cord transmission. These pathways begin in the midbrain and change the pain signals relayed by spinal cord signals. Descending pathways of significant importance begin in the Periaqueductal Gray- an area rich in μ and κ receptors- and project to the medulla and brain stem. Inhibition of the medulla and brain stem produces an additional analgesic effect because their neurons descend into the spinal cord, ultimately inhibiting cell firing.

Narcotics inhibit pain more effectively than any of the other classes of analgesics, however, the extent of their side effects limit their use. Less serious side effects include nausea, vomiting, drowsiness, and constipation (Minneman, 2005). Serious side effects

include respiratory depression, tolerance, dependency, and addiction (Minneman, 2005). According to the National Institute on Drug Abuse, 4.7 million Americans used prescription pain relievers nonmedically in 2005. This is an alarming statistic that explains the need to discover drugs that are just as effective as narcotics in relieving pain, but without the side effects. Hence, research efforts are ever increasing in search of alternative sources, synthetic or natural, of potent analgesics. One natural source that gained wide interest is the plant *Mitragyna speciosa* Korth.

E. Mitragynine

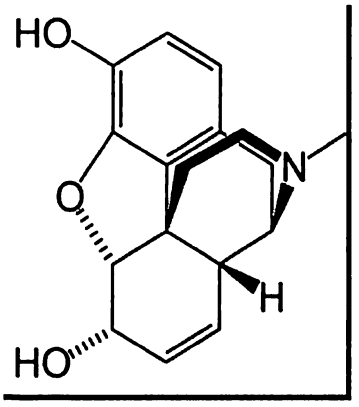
Mitragyna speciosa Korth (Rubiaceae) is a plant commonly found in Thailand and other Southeast Asian countries. Natives traditionally used its leaves for their opium-like and coca-like stimulant effect to fight fatigue and improve tolerance to hard work under the sun. The leaves have also been used as an opium substitute and for weaning addicts off morphine (Matsumoto, 1996).

In the 1960's, the Chelsea group in the U.K. isolated a significant number of indole alkaloids from the plant species native to Thailand. Mitragynine (Fig. 2B) was found to be the major constituent, accounting for 66.2% of the leaves constituents (Takayama, 2004). A new alkaloid, 7, α-hydroxy-7H-mitragynine (Fig. 2C), was later isolated as a minor constituent (2.0%) (Matsumoto, 1996).

Both mitragynine and 7, α-hydroxy-7H-mitragynine are thought to be responsible for the plants' antinociceptive activity, and both compounds demonstrate high selectivity for the μ-opioid receptor subtype (Matsumoto, 1996). It is believed that a supraspinal opioid mechanism is partly involved in the analgesic activity of mitragynine (Takayama, 2004). In a study by Macko (1972), mitragynine suppressed the cough reflex, did not

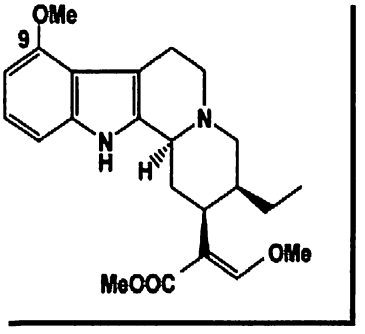
have an effect on the respiratory rate, and failed to produce toxicity at levels comparable to those of opiates in three different species of animals (Macko, 1972). In much of the previous research, the routes of administration of mitragynine were intraperitoneal (i.p.), intracerbroventrical (i.c.v.), oral, and subcutaneous. Interestingly, mitragynine is structurally different from classical opioid receptor ligands and may provide lead compounds that act as opioid analgesics without the traditional side effects associated with opiates.

A.



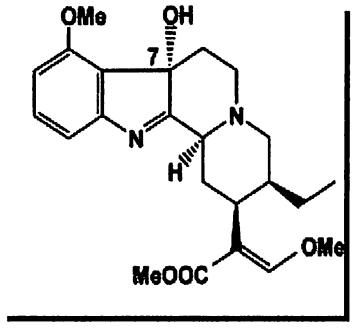
Morphine

B.



Mitragynine

C.



7, α-hydroxy-7H-mitragynine

Figure 2 The chemical structures of morphine (A), mitragynine (B), and 7, α-hydroxy-7H-mitragynine (C).

III. SPECIFIC AIMS

The purpose of this study was to pharmacologically evaluate a series of mitragynine analogs for their antinociceptive action in an effort to determine the structural features responsible for analgesic activity. In a study by Takayama (2004), researchers compared the analgesic activity of morphine to the major and minor constituents of *Mitragyna speciosa* Korth (Rubiaceae). In this study, two constituents, mitragynine and 7, α-hydroxy-7H-mitragynine, significantly increased analgesic latency. According to the results of the Takayama study, the analgesic activity of mitragynine was ¼ the potency of morphine. However, 7, α-hydroxy-7H-mitragynine, an analog of mitragynine, increased the analgesic latency nearly 13 times more than morphine. Takayama concluded that addition and/or substitution of specific chemical groups on mitragynine may increase the compounds analgesic potency. In the present study, analogs of mitragynine were synthesized and tested in vivo to determine analgesic activity. The analgesic activity or inactivity of these newly synthesized analogs may suggest which chemical groups or atoms are necessary for mitragyine's analgesic potency.

IV. MATERIALS AND METHODS

A. Subjects

For all experiments, male Swiss Webster mice (25-30 g, Harlan, Indianapolis, IN, USA) were used. The mice were housed in groups of five with a 12 h light/12 h dark cycle. Food and water were provided *ad libitum*. All mice were randomly selected for each treatment group (n = 10 per group). All procedures involving animals were performed according to the guidelines approved by the Institutional Animal Care and Use Committee.

B. Drugs

Morphine Sulfate was obtained from Sigma-Aldrich (St. Louis, MO, USA) and was dissolved in saline (0.9% NaCl). Naloxone hydrocholride was obtained from Tocris Bioscience (Ellisville, MO, USA) and was dissolved in saline (0.9% NaCl). Mitragynine was isolated by Dr. Christopher McCurdy and Jessica Adkins at the Medicinal Chemistry Department, School of Pharmacy, University of Mississisippi, and was dissolved as the hydrochloride salt in saline (0.9% NaCl). Compounds MC183, MC186, and MC187 were synthesized by Dr. Christopher McCurdy and J. Cui and provided as hydrochloride salts. Compound MC183 was dissolved in 2% DMSO, and Compounds MC186 and MC187 were dissolved in (0.9% NaCl).

C. Tail-flick Studies

The tail-flick assay (Fig. 3) characterizes thermal nociception. A tail-flick apparatus that integrates thermal nociception and an automated response timer was used (IITC Inc. Life Science, Woodland Hills, CA, USA). Mice were restrained individually

in a plexiglass restrainer, allowed to calm down for 1 minute, and the tail was positioned in a groove that marks the position where a thermal stimulus is initiated. A timer was started along with the onset of the stimulus. The timer automatically shuts off when the animal flicks its tail. The mean of two trials measured 20-30s apart was taken for each animal. A cut-off time of 15 s was used to minimize the risk of tissue damage. Animals received either vehicle (saline or 2% DMSO), morphine (5, 10, 15, 20 mg/kg), mitragynine (10, 20, 30 mg/kg), MC183 (30 mg/kg), MC186 (30 mg/kg), or MC187 (30 mg/kg) intraperitoneally. Tailflick tests were then conducted at 0, 10, 20, 30, 60, and 120 min following drug administration. Measurements taken at time 0 were considered the baseline latencies.

D. Hotplate Studies

The hotplate assay (Fig. 4) was used to assess thermal nociception at both spinal and supraspinal levels. Twenty four hours prior to testing, mice received one apparatus habituation trial (15 min) in which they are placed inside the acrylic enclosure with the hotplate (IITC Inc. Life Science, Woodland Hills, CA, USA) surface temperature maintained at room temperature. Nociceptive tests (hotplate temperature = 52 °C) were conducted with a manual timer started when all four paws of the animal made contact with the hotplate floor. Latency to flutter or lick a hindpaw served as the nociceptive measure. A 45 s cut-off time was used to minimize tissue damage. Baseline hotplate latency was measured for each animal prior to drug administration. Animals then received either vehicle (saline or 2% DMSO), morphine (5, 10, 15, 20 mg/kg), mitragynine (10, 20, 30 mg/kg), MC183 (30 mg/kg), MC186 (30 mg/kg), or MC187 (30

mg/kg) intraperitoneally. Hotplate tests were then conducted at 10, 20, 30, 60, and 120 min following drug administration.

Animals pretreated with Naloxone had their baseline readings recorded first. Following the baseline reading, the animals were injected with 10 mg/kg Naloxone and given a 15 minute latency period. At 15 minutes, the animals were injected with Morphine Sulfate (20 mg/kg) or Mitragynine (30 mg/kg) and given another 30 minute latency period. After this 30 minute latency period the final hotplate reading was recorded.

Antinociception in hotplate and tailflick tests was quantified as the maximum possible effect percent (MPE %) using the following formula:

$$\%\,\mathbf{MPE} = \frac{\textbf{(postdrug latency)- (baseline latency)}}{\textbf{(cut off time) – (baseline latency)}} \times 100$$

E. Statistical Analysis

The data are expressed as the mean ± s.e.m. Statistical analysis was performed using a one-way analysis of variance followed by Tukey's test for multiple comparisons or Dunnett's test for vehicle control comparisons. The student t-test was used to compare the groups pretreated with naloxone versus the morphine or mitragynine treated groups. A p value < 0.05 was considered statistically significant for all analyses.

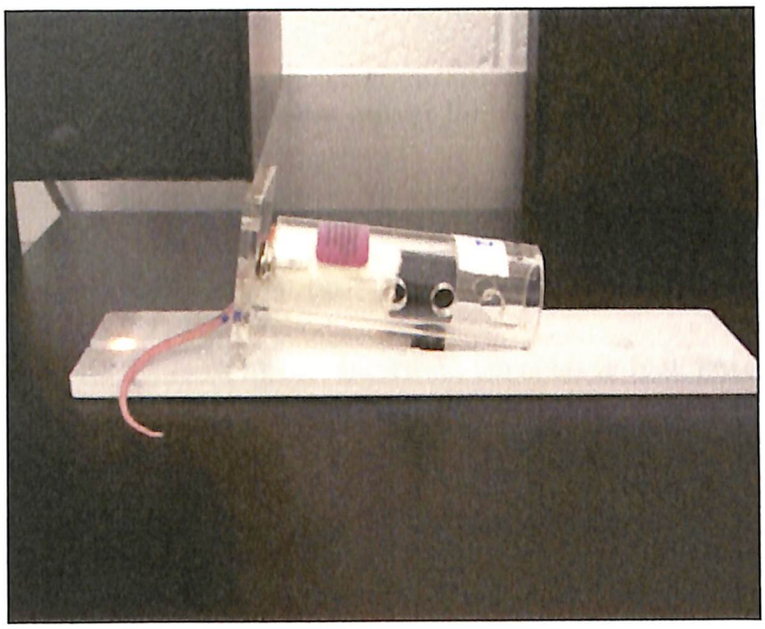


Figure 3 Photograph of the tailflick apparatus.

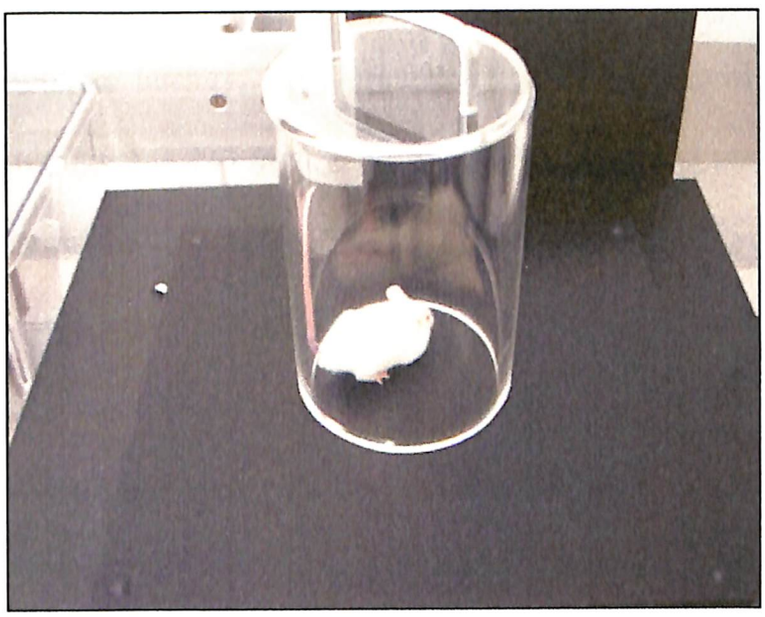


Figure 4 Photograph of the hotplate apparatus.

V. RESULTS

Time and dose response curves for morphine in the tailflick and hotplate tests

As shown in Fig. 5A and Fig. 5B, when morphine (5-20 mg/kg) was administered i.p., it significantly prolonged the nociceptive latency responses of the tailflick and hotplate tests in mice. Increased latencies in both tests corresponded to increased doses. Morphine appeared to have its maximal effect at 60 minutes (20 mg/kg) in the tailflick test and 30 minutes (20 mg/kg) in the hotplate test with a maximum %MPE of 83.04% and 89.12%, respectively.

Time and dose response curves for mitragynine in the tailflick and hotplate tests

When mitragynine (10-30 mg/kg) was administered i.p., it did not significantly prolong the nociceptive latency responses of the tailflick test in the mice, as shown in Fig. 6A, In contrast, mitragynine caused a significant dose-dependent increase in the hotplate latency. In the current study, the analgesic potency of the highest dose of mitragynine (30 mg/kg, 53.10% MPE) was about one-half that of the highest dose of morphine (20 mg/kg, 89.12% MPE) in the hotplate test. Again, the maximal effect of mitragynine (30 mg/kg) was at 30 minutes. Therefore, in Fig. 7, the nociceptive responses of different doses at 30 minutes after i.p. administration of morphine and mitragynine were compared in both tests.

Attenuation of antinociceptive action of morphine and mitragynine by naloxone

In Fig. 8, the antinociceptive actions of the i.p. injections of morphine (20 mg/kg) observed in both the hotplate and tailflick tests were significantly attenuated by pretreatment with naloxone (10 mg/kg), a preferential μ-opioid antagonist (Takayama,

2004). Naloxone also attenuated the antinociceptive effects of mitragynine (30 mg/kg) in the hotplate test (shown in Fig. 9).

Evaluation of synthetic analogs of mitragynine in the tailflick and hotplate tests

As shown in Fig. 10-12, the synthetic analogs of mitragynine, MC183 (30 mg/kg), MC186 (30 mg/kg), and MC187 (30 mg/kg) failed to show a significant increase in tailflick or hotplate latencies when compared to mitragynine (30 mg/kg).

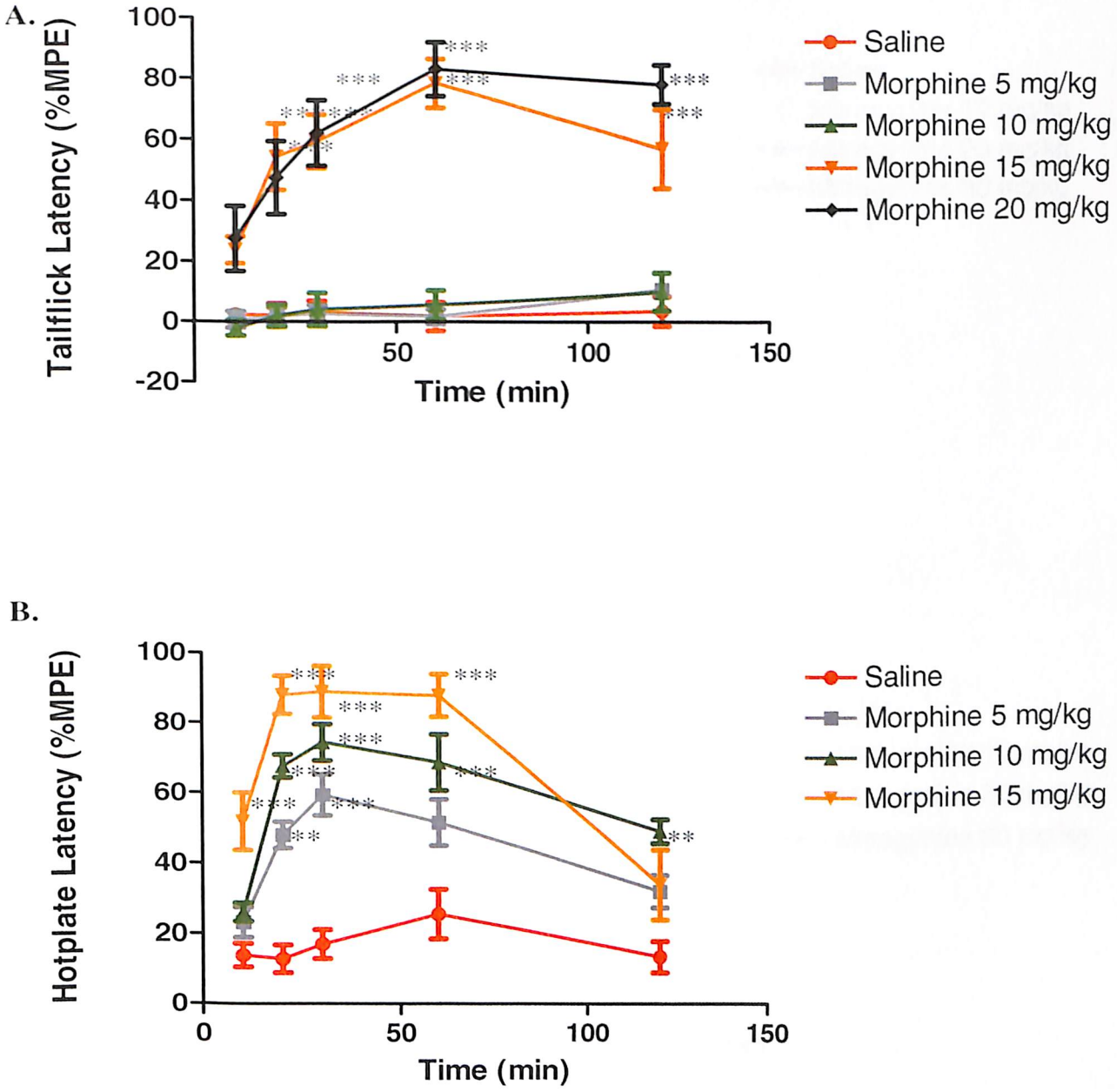
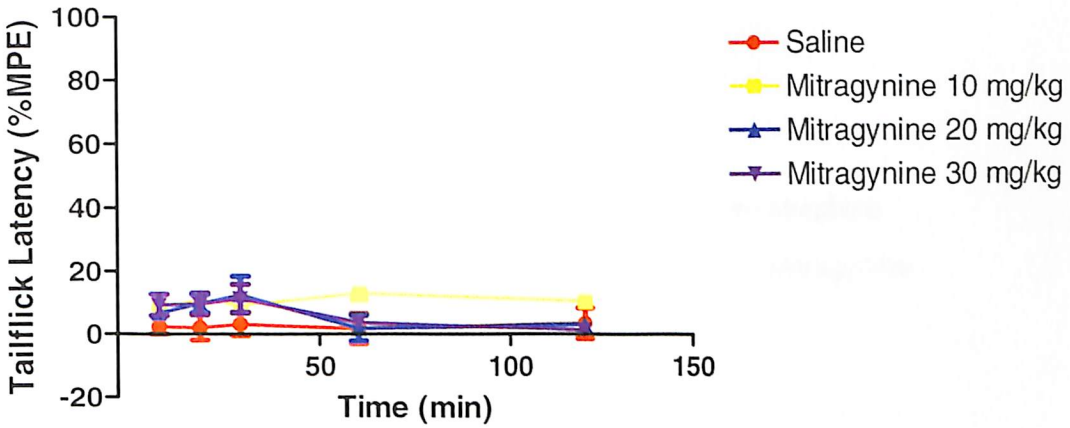


Figure 5 Time and Dose Response Curves for Morphine in the Tailflick (A) and Hotplate (B) Tests. Morphine showed a steep dose-response antinociceptive action in the tailflick assay as well as the hotplate assay. The results suggest a spinal and supraspinal mechanism mediating the effect. ** $p<0.01$ versus saline, *** $p< 0.001$ versus saline.

A.



B.

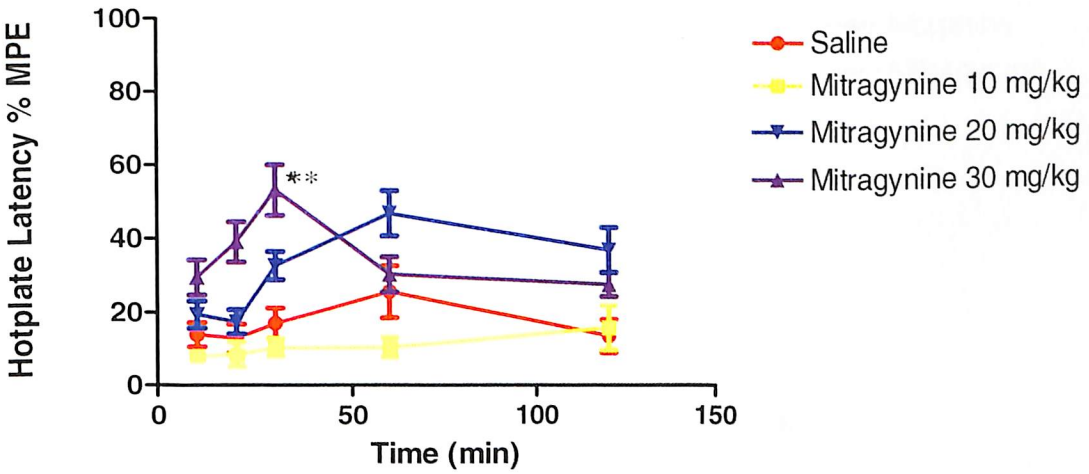
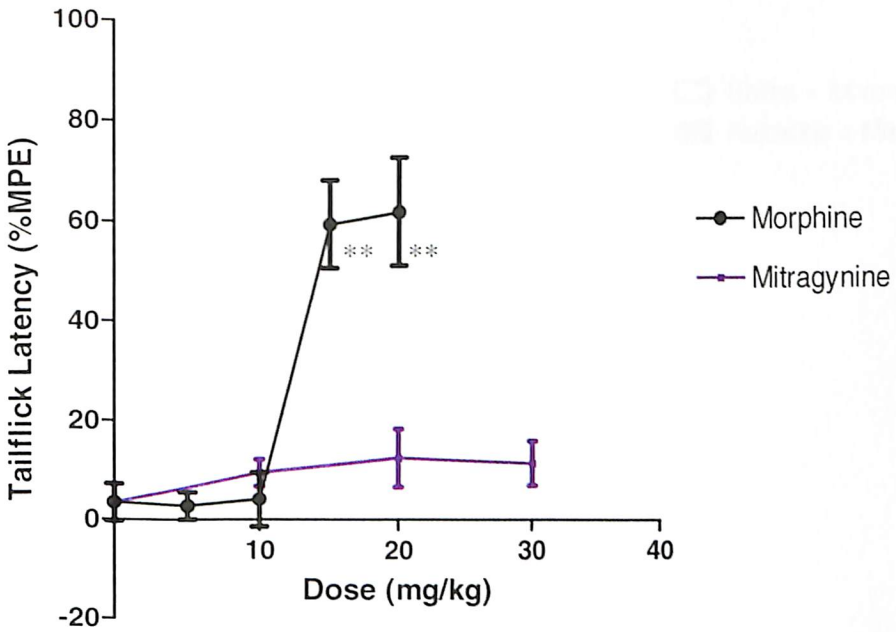


Figure 6 Time and Dose Response Curves for Mitragynine in the Tailflick (A) and Hotplate (B) Tests. Mitragynine showed dose-dependent antinociceptive action in the hotplate assay while no significant effect was observed in the tailflick assay. The results suggest a supraspinal mechanism mediating the effect. * $p<0.05$ versus saline, ** $p<0.01$ versus saline, *** $p< 0.001$ versus saline.

A.



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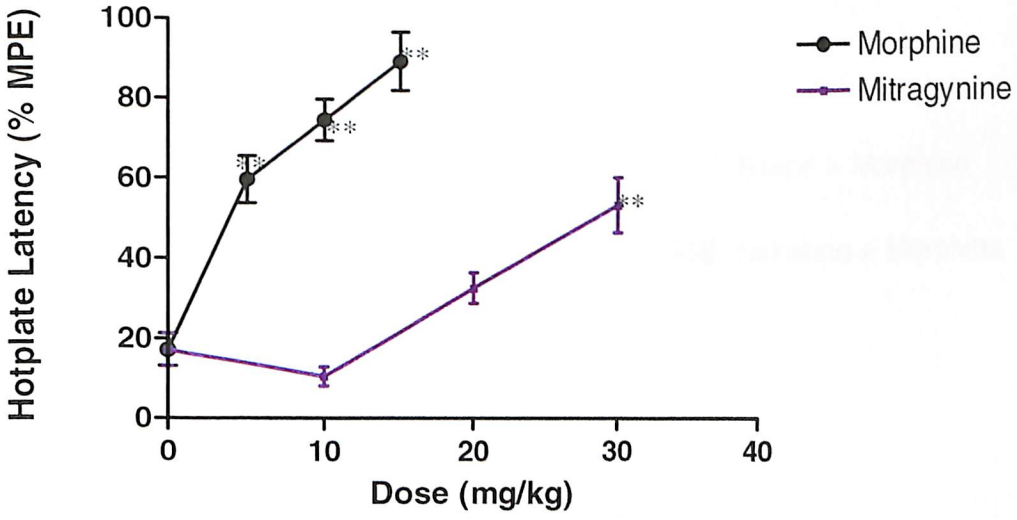
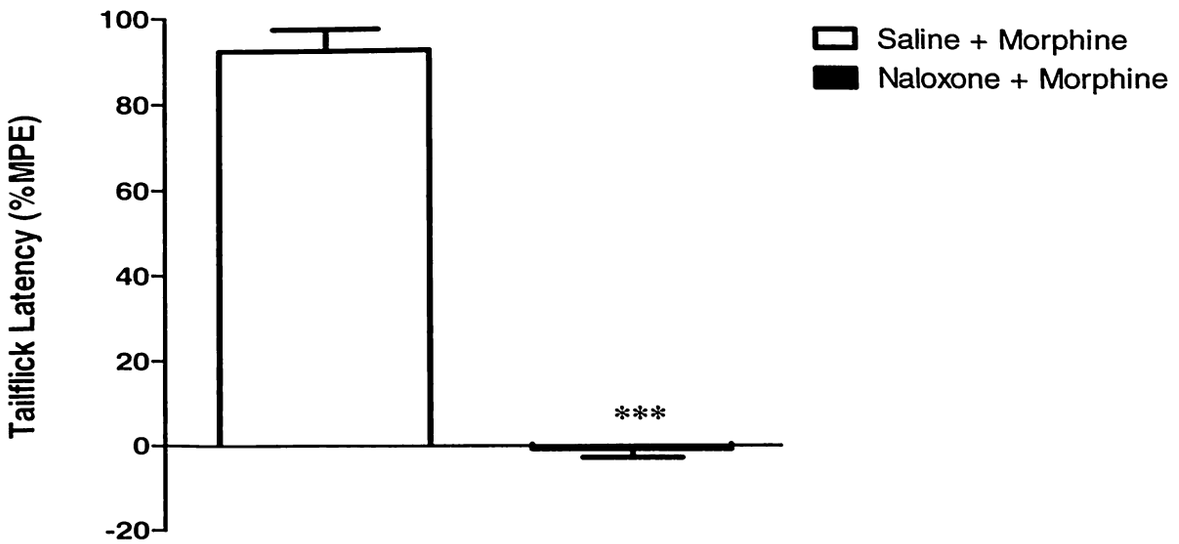


Figure 7 Dose Response Curves for Morphine and Mitragynine at 30 minutes in the Tailflick (A) and Hotplate (B) Tests. Mitragynine demonstrated no significant antinociceptive action in the tailflick assay. However, Mitragynine demonstrated antinociceptive action in the hotplate assay but was far less potent than Morphine. ** $p<0.01$ versus saline

A.



B.

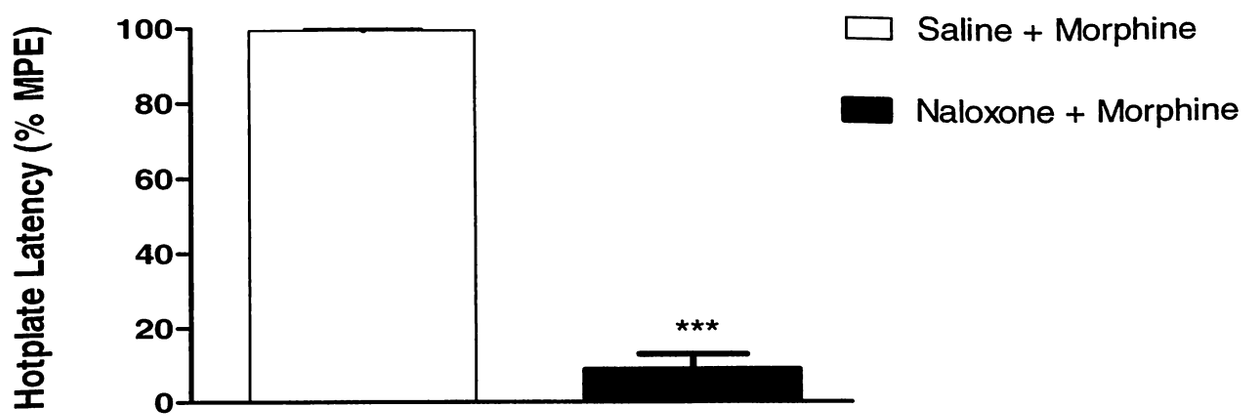
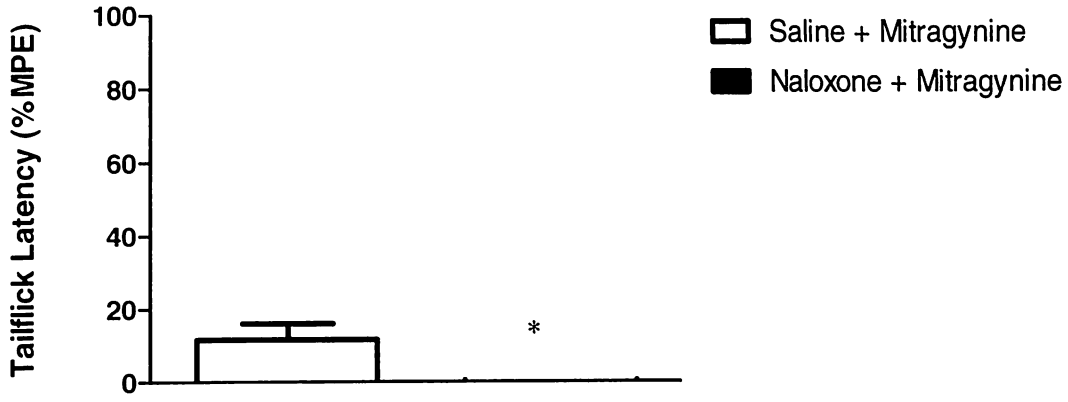


Figure 8 Attenuation of Antinociceptive Action of 20 mg/kg Morphine by 10 mg/kg Naloxone. The antinociceptive effects of Morphine (20 mg/kg i.p.) in the tailflick (A) and hotplate (B) tests were blocked by pretreatment with the opioid receptor antagonist Naloxone. *** $p < 0.001$ versus morphine.

A.



B.

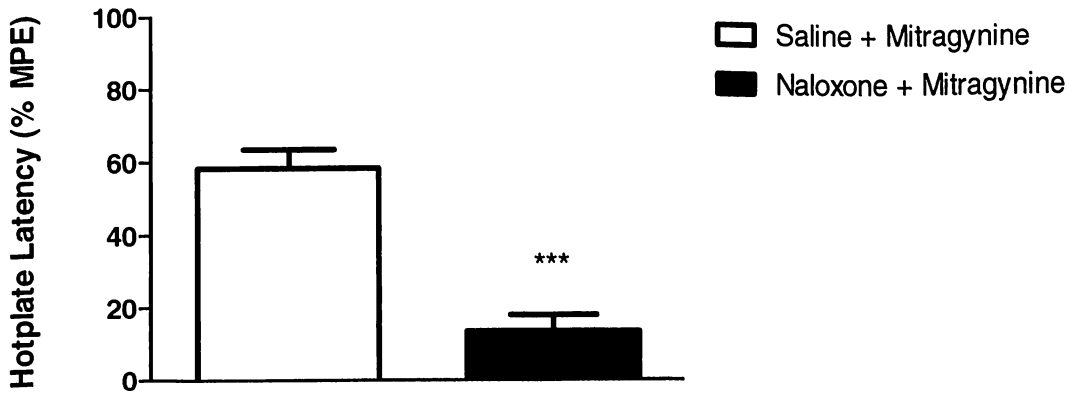
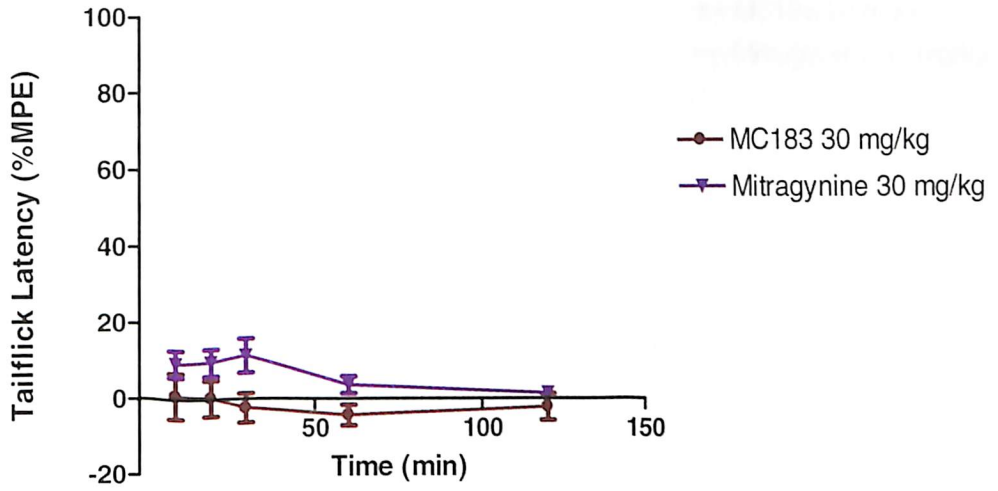


Figure 9 Attenuation of Antinociceptive Action of 30 mg/kg Mitragynine by 10 mg/kg Naloxone. The antinociceptive effects of Mitragynine in the tailflick (A) and hotplate (B) tests were blocked by pretreatment with the opioid receptor antagonist Naloxone. * $p<0.05$ versus mitragynine, *** $p< 0.001$ versus mitragyine.

A.



B.

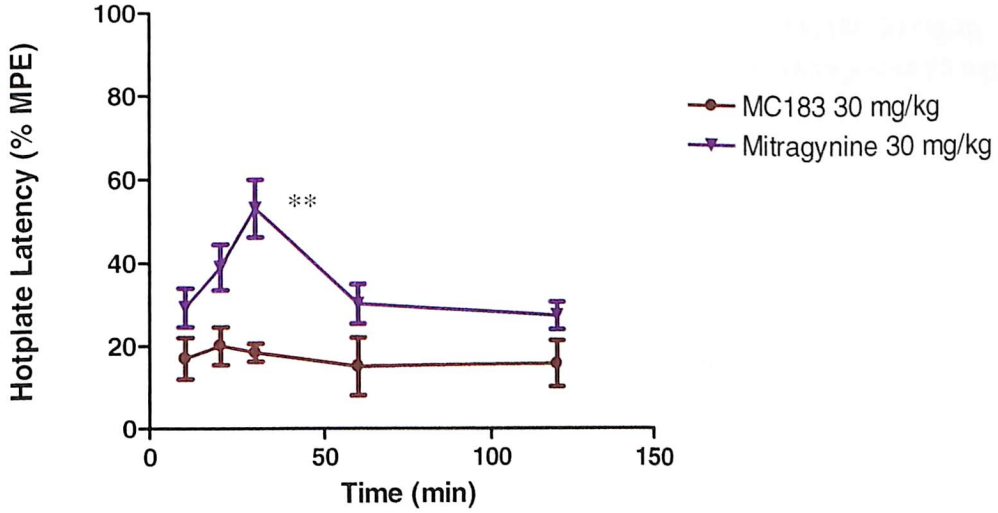
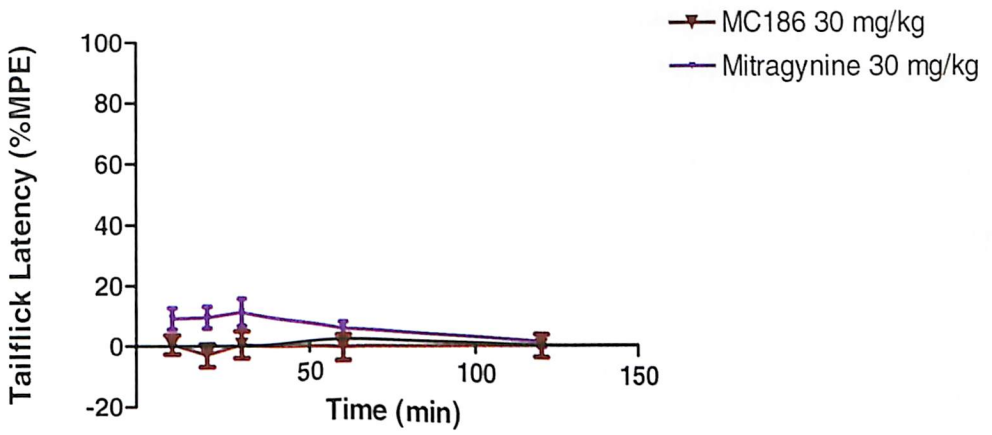


Figure 10 Evaluation of MC183 and Mitragynine in the Tailflick (A) and Hotplate (B) Tests. Compound MC183 at 30 mg/kg did not show any analgesic effect in the hotplate or tailflick assay. ** $p<0.01$ versus saline.

A.



B.

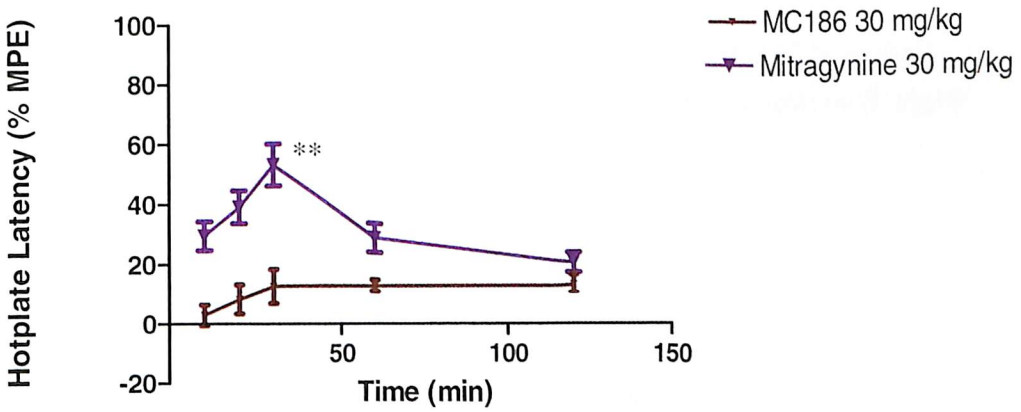
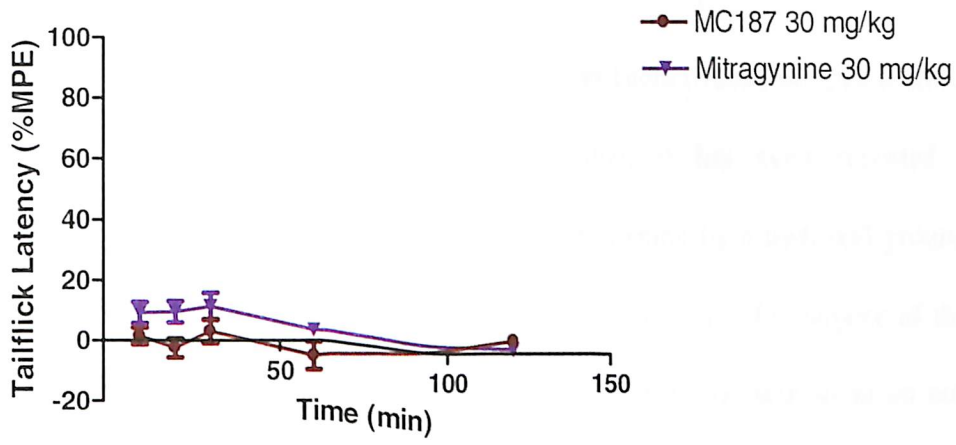


Figure 11 Evaluation of MC186 and Mitragynine in the Tailflick (A) and Hotplate (B) Tests. Compound MC186 at 30 mg/kg did not show any analgesic effect in the hotplate or tailflick assay. ** $p<0.01$ versus saline.

A.



B.

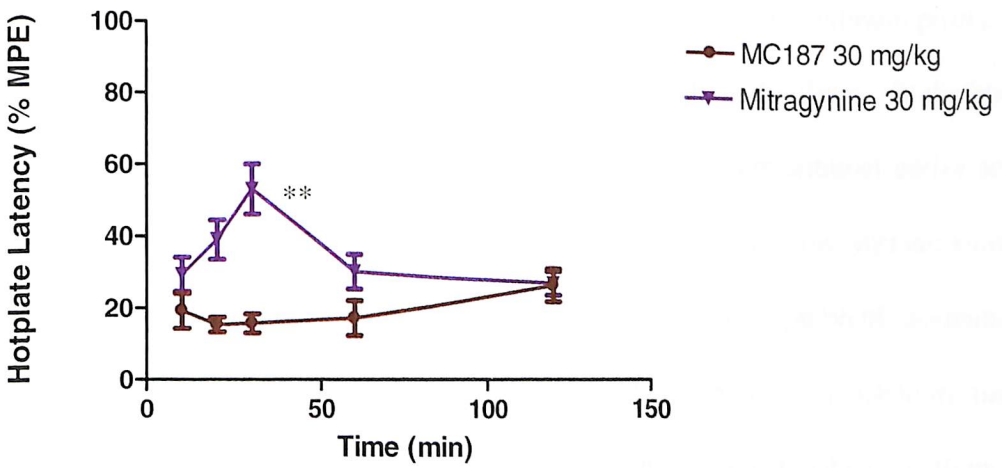


Figure 12 Evaluation of MC187 and Mitragynine in the Tailflick (A) and Hotplate (B) Tests. Compound MC187 at 30 mg/kg did not show any analgesic effect in the hotplate or tailflick assay. ** $p<0.01$ versus saline.

VI. DISCUSSION

Previous studies demonstrated an increase in latency in the tail-pinch and hotplate tests after administration of mitragynine. Also, it has been reported that 7-hydroxymitragynine, which only differs from mitragynine by a hydroxyl group, has an analgesic potency thirteen times that of morphine. Therefore, the purpose of this study was to evaluate a series of mitragynine analogs for analgesic activity in an attempt to determine the pharmocophoric groups responsible for action.

In the tailflick test, morphine (5- 20 mg/ kg i.p.) exerted a dose-dependent increase in latency that reached its maximum activity at approximately 20-60 minutes following the injection. In a study by Matsumoto (1996), morphine (1- 3 mg/ kg i.c.v.) demonstrated dose-dependent antinociceptive activity that peaked approximately 15 minutes after the injection. The differences in the time at which maximum potency was observed could be due to the administration routes as well as the doses. In the hotplate test, morphine (5- 20 mg/ kg i.p.) demonstrated dose-dependent antinociceptive activity that had a maximal effect at 20-60 minutes post injection. These findings are similar to the findings published by Andoh (2007) in which single administration of morphine (10-30 mg/ kg oral) dose-dependently inhibited thermal hyperalgesia and reached its maximal effect at 30 minutes post injection. The opioid receptor antagonist, naloxone (10 mg/ kg), significantly attenuated the antinociceptive activity of morphine in both the tailflick and hotplate test, which corresponds to previously published data (Matsumoto, 1996; Takayama, 2004).

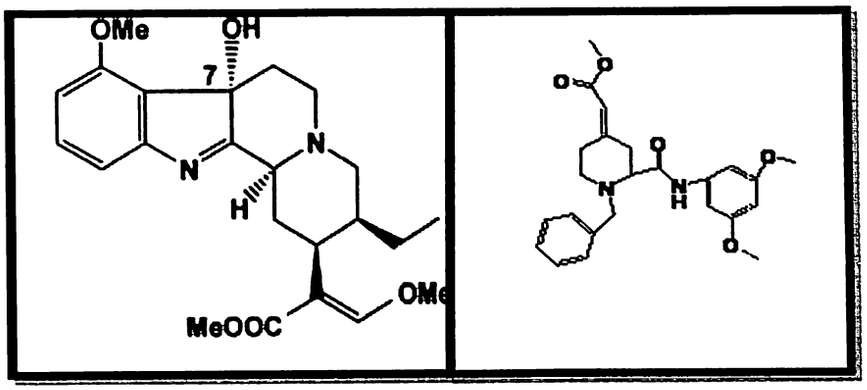
In the present study, mitragynine (10- 30 mg/ kg i.p.) did not successfully lead to an increase in antinociceptive activity in the tailflick test. These results were not typical

of published literature on mitragynine. In a study by Matsumoto (1996), administration of mitragynine (5- 30 mg/ kg) resulted in a dose-dependent increase in latency that demonstrated a maximal effect at 15- 45 minutes following injection. These results applied to both the tail-pinch and hotplate tests (Matsumoto, 1996). In this study, the tailflick test was utilized as opposed to the tail-pinch test used by Matsumoto, and the route of injection was i.p. as opposed to supraspinally in the Matsumoto study. Also, a different mice strain was used in the present study, and these changes may account for the differences in results. In addition, a higher dose of mitragynine should be examined to determine if, in fact, it lacks activity in the tailflick test. In the hotplate test, administration of mitragynine (10- 30 mg/ kg i.p.) exerted dose-dependent antinociceptive activity with a maximal effect at 20- 60 minutes after injection. These findings agree with data published by Matsumoto (1996) in which mitragynine (5- 30 mg/ kg i.p.) demonstrated dose-dependent antinociceptive activity with a maximal effect at 15- 45 minutes post injection. Also, pretreatment with the opioid receptor antagonist, naloxone (10 mg/ kg i.p.), attenuated the antinociceptive activity in the tailflick and hotplate tests. These results are in accordance with the data reported by Matsumoto et al. (1996). However, it is important to note that naloxone was administered s.c. and i.c.v. in the Matsumoto study. These findings indicate the involvement of opioid receptors in the antinociceptive activity of mitragynine.

The analogs of mitragynine (Fig. 14) used in the present study were called MC183, MC186, and MC187, and were synthesized by J. Cui under the direction of Dr. Christopher McCurdy. In each of the structures, the 4-ring structure was compromised in order to determine if it was essential for mitragynine's analgesic activity. The analogs

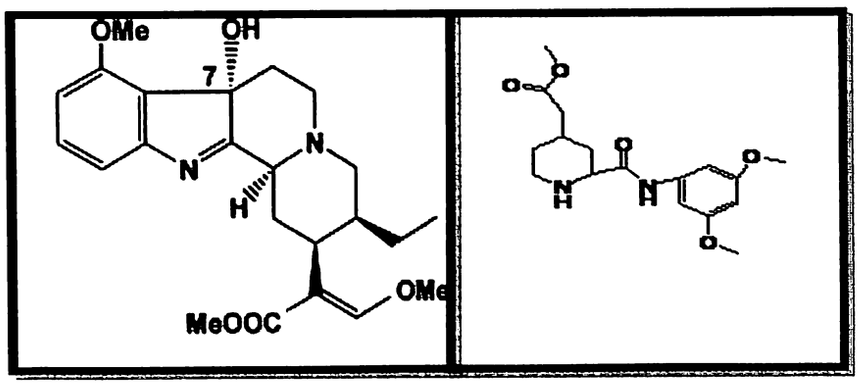
had not previously been synthesized or tested. Therefore, the dose administered in the tailflick and hotplates tests was 30 mg/kg dose, which was the dose that was significantly effective for mitragynine in the hotplate test. It is possible that these compounds could have been effective at much higher doses. However, at 30 mg/kg, the synthesized compounds failed to show statistical significance in the tailflick or hotplate latencies when compared to mitragynine (30 mg/kg). According to Takayama's findings (2004), the methoxyl group at the C-9 position is required for antinociceptive activity. Also, changing the functional group at this position shifted the activity of the compound from an agonist to an antagonist (Takayama, 2004). In addition, Takayama concluded that the N_b- lone electron pair was necessary for the antinociceptive activity of mitragynine.

A.



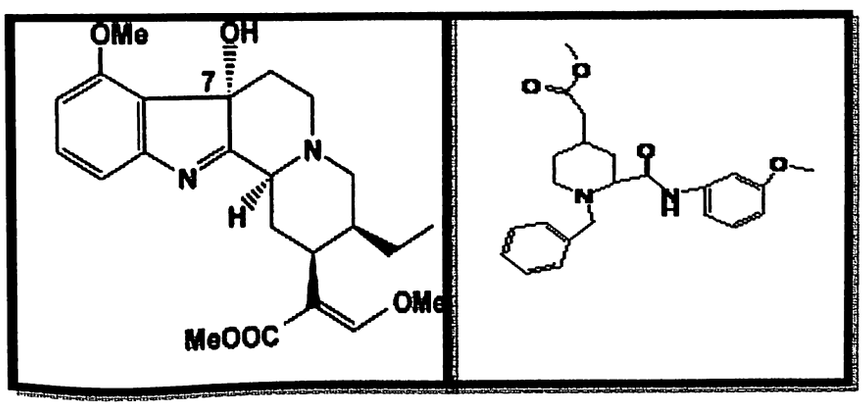
Mitragynine MC183

B.



Mitragynine MC186

C.



Mitragynine MC187

Figure 13 The chemical structure of mitragynine next to the synthetic analogs MC183 (A), MC186 (B), and MC187 (C).

VII. CONCLUSION

In conclusion, the ring structure of mitragynine must remain intact if an analgesic effect is desired. In a previous study conducted by Takayama (2004), it was suggested that 7-hydroxymitragynine, which can be isolated from the plant or synthesized by the addition of a hydroxyl group at the 7^{th} carbon position of mitragynine, is in fact, the active ingredient of the plant. Like Macko (1972), Takayama (2004) reported fewer side effects regardless of the dose of mitragynine as well as its derivatives. These results are uncommon for opioid compounds. Current findings suggest a positive future for mitragynine in the development of new compounds to treat pain. Future plans for research include synthesizing 7-hydroxymitragynine, testing its antinociceptive activity in the tailflick and hotplate assays, and determining its mechanism of action. After comparing the results to those of morphine and mitragynine, additional tests could be conducted to determine if the compound induces cocoa-like stimulant effects and other possible side effects that are typical of opioids.

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