EFFECT OF ATIPAMEZOLE ON THE ANTINOCICEPTIVE PROPERTIES OF BUTORPHANOL AND BUPRENOHRPHINE FOLLOWING KETAMINE AND DEXMEDETOMIDINE ANESTHESIA IN FEMALE C57BL/6J MICE

A Thesis in Laboratory Animal Medicine

by Jenelle M. Tretter

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ABSTRACT

The combination of ketamine and dexmedetomidine is often used for anesthesia of rats and mice in laboratory research. As an α2-adrenoceptor agonist, the sedative effects of dexmedetomidine can be reversed by administration of an α2-adrenoceptor antagonist, such as atipamezole, to speed anesthetic recovery. Opioids are often administered postoperatively for their potent analgesic effects. A previous study has shown that atipamezole may antagonize the antinociceptive effects of opioids in rats, but literature is lacking regarding the effects of atipamezole on butorphanol and buprenorphine in mice. If the antinociceptive effects of opioid analgesics are inadvertently attenuated by atipamezole, poor postoperative analgesia could have a significant impact on animal welfare, regulatory compliance and research results. A six-part study was designed to investigate the effects of atipamezole administered before butorphanol or buprenorphine, on tail-flick latency and also following ketamine-dexmedetomidine anesthesia in female C57BL/6J mice. Nociception was evaluated using the tail-flick test at 15, 30, 60, 90, 120 and 150 minutes following drug treatment. Atipamezole alone had no significant effect on tail-flick latency and no significant effect on the antinociceptive properties of butorphanol or buprenorphine in mice. Following ketamine-dexmedetomidine anesthesia, there were no significant differences in tail-flick latency values between the atipamezole and atipamezole plus butorphanol groups. Similarly, there were no significant differences in tail-flick latencies between the atipamezole and atipamezole plus buprenorphine groups following anesthesia, except at 90 and 150 minutes after treatment. These results suggest that the analgesic effects of
butorphanol and buprenorphine are not affected by the reversal agent, atipamezole.

However, buprenorphine should be given prior to surgery to provide adequate postoperative analgesia in C57BL/6J mice. This recommendation is supported by the late onset of buprenorphine-induced analgesia, as evidenced by increased tail-flick latencies at 90 minutes following treatment.
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<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>AAALAC</td>
<td>Association for Assessment and Accreditation of Laboratory Animal Care, International</td>
</tr>
<tr>
<td>Atip</td>
<td>Atipamezole</td>
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<tr>
<td>Ax</td>
<td>Anesthesia</td>
</tr>
<tr>
<td>But</td>
<td>Butorphanol</td>
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<tr>
<td>Bup</td>
<td>Buprenorphine</td>
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<tr>
<td>cm</td>
<td>Centimeter</td>
</tr>
<tr>
<td>CNS</td>
<td>Central nervous system</td>
</tr>
<tr>
<td>DEA</td>
<td>Drug Enforcement Administration</td>
</tr>
<tr>
<td>g</td>
<td>Gram</td>
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<tr>
<td>hr</td>
<td>Hour</td>
</tr>
<tr>
<td>IACUC</td>
<td>Institutional Animal Care and Use Committee</td>
</tr>
<tr>
<td>IP</td>
<td>Intraperitoneal</td>
</tr>
<tr>
<td>K-D</td>
<td>Ketamine and dexmedetomidine</td>
</tr>
<tr>
<td>kg</td>
<td>Kilogram</td>
</tr>
<tr>
<td>LORR</td>
<td>Loss of righting reflex</td>
</tr>
<tr>
<td>min</td>
<td>Minutes</td>
</tr>
<tr>
<td>mg</td>
<td>Milligram</td>
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<tr>
<td>ml</td>
<td>Milliliter</td>
</tr>
<tr>
<td>NMDA</td>
<td>N-methyl-D-aspartate</td>
</tr>
<tr>
<td>NSAID</td>
<td>Nonsteroidal anti-inflammatory</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Description</td>
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<td>--------------</td>
<td>------------------------------</td>
</tr>
<tr>
<td>RORR</td>
<td>Return of righting reflex</td>
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<tr>
<td>sec</td>
<td>Seconds</td>
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<tr>
<td>TFL</td>
<td>Tail-flick latency</td>
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<tr>
<td>α2</td>
<td>Alpha-2</td>
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<tr>
<td>κ</td>
<td>Kappa</td>
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Chapter 1

Introduction

The alleviation of pain and distress in animals undergoing experimental procedures is necessary not only for ethical reasons, but also in order to prevent potential adverse effects on experimental results. Pain is associated with a variety of physiological changes and has been shown to have specific effects on wound healing, metabolism, behavior, and on immune, pulmonary, gastrointestinal, cardiac and urinary tract function. Furthermore, individuals involved in scientific research have regulatory obligations to minimize pain and distress in research animals. Analgesic therapies are frequently administered pre-, peri- and postoperatively in laboratory animal species for pain management. Pain management supports the refinement principle of animal research, which aims to reduce the incidence or severity of distress experienced by laboratory animals. If the antinociceptive effects of a particular analgesic are inadvertently attenuated by the administration of another drug, poor post-procedural pain management could have a significant impact on animal welfare, regulatory compliance and scientific results.

In rodents, atipamezole is often used to hasten recovery from anesthesia, while opioids are commonly administered postoperatively for their potent analgesic effects. The results of a previous study demonstrated that atipamezole attenuated the analgesic effects of the opiate, butorphanol, in adult male Sprague-Dawley rats. Although the mouse (Mus musculus) is currently the most popular laboratory animal used in biomedical
research, an extensive literature search yielded no investigations of the potential effects of atipamezole on the antinociceptive effects of opioids in mice. This gap in the literature is important to address, as attenuated analgesia could have substantial consequences on research. Therefore, the present study was performed to investigate whether atipamezole would alter the antinociceptive effects of butorphanol and buprenorphine in C57BL/6J mice.

The combination of ketamine and dexmedetomidine is often used for anesthesia of rats and mice. Ketamine is a noncompetitive N-methyl-D-aspartate (NMDA) receptor antagonist. Ketamine is considered a potent analgesic that blocks pain impulses to thalamic and cortical areas of the brain. Due to its wide margin of safety, low cost and ease of administration, ketamine is commonly used in veterinary medicine. As a sympathetic nervous system stimulant, ketamine has positive inotropic and chronotropic effects. High doses of the drug produce dissociative anesthesia and behavioral changes that limit its efficacy when used as a sole anesthetic agent. Additionally, ketamine administered alone can cause muscle rigidity, increased salivation leading to potential airway obstruction and hypertension. Because ketamine is a cardiovascular stimulant, it is often used in combination with other anesthetic agents, such as α2-adrenoceptor agonists, for general anesthesia in laboratory rodents. Furthermore, α2-adrenoceptor agonists are frequently combined with ketamine to provide muscle relaxation and smooth recovery from ketamine anesthesia.

Dexmedetomidine is an α2-adrenoceptor agonist that stimulates central receptors located in the spinal cord and supraspinal α2-autoreceptors within the brainstem to induce sedative and analgesic effects. In addition to sedation and analgesia, α2-adrenoceptor
agonists produce muscle relaxation, anxiolysis and potent anesthetic sparing effects. Dexmedetomidine is the dextrorotatory enantiomer of the α2-adrenergic agonist, medetomidine and is approximately two times more potent than medetomidine.\textsuperscript{18} Xylazine was the first α2-adrenergic agonist used in veterinary medicine and is the least potent sedative and analgesic of any of the α2-adrenergic agonists.\textsuperscript{19} Dexmedetomidine is much more specific than xylazine for α2 receptors versus α1 receptors.\textsuperscript{18} Common side effects of α2-adrenoceptor agonists include bradycardia, hypotension, respiratory depression and hypothermia. The anesthetic combination of ketamine and dexmedetomidine is commonly used in many laboratory animal species, as the stimulatory effects of ketamine offset the depressant effects of dexmedetomidine.\textsuperscript{16} Moreover, dexmedetomidine offsets muscle rigidity produced by ketamine and enhances overall analgesia. The advantage of using an α2-adrenoceptor agonist, such as dexmedetomidine, is the opportunity to reverse the sedative and cardiovascular effects of the drug with an α2-adrenoceptor antagonist, such as atipamezole.

Atipamezole is a highly selective α2-adrenoceptor antagonist that is used following medetomidine or dexmedetomidine administration to reverse the effects of the α2-adrenoceptor agonist on central and peripheral receptors to restore cardiovascular and respiratory function.\textsuperscript{15} Reversal of anesthesia following a surgical procedure is advantageous in mice and rats by hastening recovery, thus reducing the risk of hypothermia, which is a considerable problem associated with anesthesia in these species.\textsuperscript{4,7,11} While atipamezole reverses the cardiovascular and sedative effects of dexmedetomidine, it also reverses the analgesic effects of the α2-adrenoceptor agonist drug.\textsuperscript{18} Without reversal of the drug, the analgesic effects of dexmedetomidine in cats are
only maintained for up to 60 minutes, which would be inadequate for postoperative analgesia.\textsuperscript{18} As a result, it is common for other analgesics, frequently opioids, to be administered postoperatively to prevent pain associated with various surgical procedures.

Two opioid drugs that are often administered to rodents for postoperative pain management include butorphanol and buprenorphine. Butorphanol exhibits partial agonist and antagonist activity at the $\mu$ opioid receptor and agonist activity at the $\kappa$ opioid receptor. Butorphanol is often used in large animal species and rats for its sedative effects because it has minimal effects on the cardiovascular system. Buprenorphine is a partial $\mu$-agonist that is widely used for its analgesic effects in mice and rats. Buprenorphine is commonly used for postoperative analgesia due to its long duration of action and minimal adverse side effects. In rats, reported side effects of buprenorphine include pica, decreased intestinal motility, respiratory depression and sedation.\textsuperscript{6}

A previous study showed that central serotonergic neurons are required for the full expression of opioid analgesia.\textsuperscript{22} Furthermore, $\kappa$-opioid analgesia is entirely dependent on supraspinal serotonergic pathways.\textsuperscript{22} Based on these findings, one group investigated the effects of a potent $\alpha_2$-antagonist on the antinociceptive properties of butorphanol in rats.\textsuperscript{14} In this study, atipamezole attenuated the analgesic effects of butorphanol in rats, suggesting that blocking $\alpha_2$-adrenoceptors may have inhibited the activity of serotonergic pathways and subsequently, butorphanol-induced analgesia.\textsuperscript{14} These findings are significant in that atipamezole used to hasten recovery may interfere with the postoperative analgesic effect of butorphanol. However, in this study, the tail-flick latency of rats was not measured after administration of atipamezole alone, allowing a potentially unidentified hyperalgesic effect of the $\alpha_2$-adrenoceptor antagonist. To our
knowledge, no studies have examined the potential effects of atipamezole on butorphanol and buprenorphine in mice.

Tests of nociception are frequently used to measure the analgesic properties of drugs. The tail-flick assay is commonly used in basic pain research to evaluate the effectiveness of various analgesics. The purpose of the current study was to assess nociception after atipamezole alone (experiment 1), pretreatment with atipamezole followed by butorphanol (experiment 2), pretreatment with atipamezole followed by low- and high-dose buprenorphine (experiments 3 and 4), butorphanol following ketamine-dexmedetomidine anesthesia with atipamezole reversal (experiment 5) and buprenorphine following ketamine-dexmedetomidine anesthesia with atipamezole reversal (experiment 6) in female C57BL/6J mice. The hypothesis to be tested was that atipamezole may inhibit the antinociceptive effects of butorphanol, but would have no effect on the antinociceptive action of buprenorphine in female C57BL/6J mice.
Chapter 2

Materials and Methods

Animals

A total of 168, 3-4 week old female C57BL/6J mice (*Mus musculus*) were utilized in this study. C57BL/6J mice were chosen for use in this project because they are the most commonly used inbred strain in pain-related studies. Mice were purchased from The Jackson Laboratory (Stock # 000664, Bar Harbor, ME). Female mice were used in order to prevent the loss of study animals as a consequence of fighting, which frequently occurs among group-housed male mice. All mice were in the weight range of 17-31 g at the time of experimentation. Mice were housed 5 mice per cage, in open-top, solid-bottom polycarbonate cages (Max75, Alternative Design, Siloam Springs, AR) with wire bar lids, corncob bedding (Teklad 7097 Corn Cob Bedding, Harlan, Frederick, MD) and nestlets (Ancare, Bellmore, NY) for environmental enrichment. Mice received *ad libitum* standard commercial rodent chow (Teklad 2018 Global 18% Protein Rodent Diet, Harlan, Frederick, MD) and municipal tap water provided in bottles. Cages were changed weekly. The animal housing room was maintained at a temperature of 70-73°F, a relative humidity of 30 to 70%, 18 air changes hourly, and on a 12:12-h light:dark cycle with lights on at 0700 and off at 1900 hours with no twilight. Mice underwent an acclimation period of 5 days before studies were initiated. Mice were housed in an AAALAC-accredited facility, in compliance with the standards set forth in the *Guide for the Care
and Use of Laboratory Animals, 8th Edition. All procedures were approved by the
Institutional Animal Care and Use Committee (IACUC) of the Pennsylvania State
University College of Medicine, M.S. Hershey Medical Center.

In order to assess the health status of the mice, outbred female Crl:CD1 (Charles
River Labs, Wilmington, MA; age 4-6 wk) mice were used as sentinels. Dirty bedding
was transferred into sentinels’ cages at each weekly cage change. All sentinels were
tested quarterly for viral, microbial, and endo- and ectoparasitic infection. Serology tests
(IDEXX RADIL Mouse Comprehensive Plus Serology Profile) were negative for mouse
hepatitis virus, minute virus of mice, mouse parvovirus, mouse norovirus, Theiler murine
encephalomyelitis virus, mouse rotavirus, Sendai virus, pneumonia virus of mice,
reovirus3, lymphocytic choriomeningitis virus, ectromelia virus, mouse adenovirus-1,
mouse adenovirus-2, polyoma virus, mouse cytomegalovirus, Encephalitozoon cuniculi,
cilia-associated respiratory bacillus, Clostridium piliforme, and Mycoplasma pulmonis.
Sentinel mice were free of endo- and ectoparasites.

Drug Preparation and Administration

The drugs used in this study were ketamine hydrochloride (Ketathesia®, Butler
Animal Health Supply, Dublin, OH), dexmedetomidine hydrochloride (Dexdomitor®,
Pfizer Animal Health, New York, NY) atipamezole hydrochloride (Antisedan®, Pfizer
Animal Health, New York, NY) butorphanol tartrate (Torbugesic®, Fort Dodge Animal
Health, Fort Dodge, IA) and buprenorphine hydrochloride (Buprenex®, Reckitt
Benckiser Pharmaceuticals, Inc., Richmond, VA). An anesthetic mixture of 50 mg/kg
ketamine and 0.5 mg/kg dexmedetomidine was prepared by diluting the drugs in sterile isotonic saline. The ketamine-dexmedetomidine anesthetic solution was dosed at 0.2 ml/10 g. Butorphanol, buprenorphine and atipamezole were diluted with sterile water for more accurate dosing of the drugs. The concentrations of the diluted drugs were: butorphanol, 0.5 mg/ml; buprenorphine, 0.015 mg/ml; and atipamezole 0.25 mg/ml. At these dilutions, all drugs were administered at an approximate volume of 0.05-0.1 ml. After the baseline tail-flick latencies were measured, the drugs were administered intraperitoneally into the caudal abdomen using a 1-ml syringe and a 25-gauge 5/8” needle. Each mouse received only one drug treatment and was euthanized at the end of the study.

**Assessment of Antinociception**

The tail-flick test was used to assess nociception using an analgesia meter (Ugo Basile, Model 37360, Schwenksville, PA). The analgesia meter (Figure 1) measures nociceptive threshold to an infrared heat source on the tail. The timer is started simultaneously when the heat stimulus is applied to the tail. When the mouse feels pain and flicks its tail, a sensor detects it, subsequently stops the timer and switches off the infrared heat. The radiant heat source has an adjustable intensity ranging from 10 to 99. An intensity setting of 60 and a cut-off time of 10 seconds were used throughout the study to prevent tissue injury to the tail. These settings were determined from a literature search to yield baseline tail-flick latencies between 2-3 seconds.  

Analgesiometric testing was performed in a dedicated procedure room separate from the animal housing room. The cages were transported to the procedure room on
acclimation days as well as the day of testing. Mice remained in their home cages with
the same cagemates during the test period. To avoid a stress response on the test day,
mice were acclimated to gentle restraint on the tail-flick unit for 3 days prior to the test
day. Each mouse was lightly restrained by hand using a disposable blue underpad for 20-
30 seconds. Gentle restraint was used to avoid induction of a stress response by holding
the mouse too tightly. An animal that is restrained too tightly may exhibit increased tail-
flick latencies or not flick the tail at all.²

The mice were habituated for 30 minutes in the procedure room on the day of
testing. Each mouse was weighed and the dorsal aspect of their tails was marked with a
non-toxic, black permanent marker in approximately 0.5 cm increments, starting 0.5 cm
from the distal end of the tail. Each tail-flick measurement was conducted at a different
position on the tail in order to prevent the tissue from becoming sensitized or desensitized
to the heat stimulus. Multiple tail-flick tests may produce tissue injury, subsequently
influencing tail-flick latencies.² Furthermore, non-pigmented regions of the tail were
avoided during tail-flick testing, as previous studies have shown that non-pigmented
regions of the C57BL/6J mouse tail significantly increase the response latency in the tail
flick assay.²¹ Each mouse was used only once for experimentation. Baseline tail-flick
latencies were measured at the first increment on the tail. Mice that did not have baseline
tail-flick latencies between 2 and 3 seconds were eliminated from the study in order to
account for variation in pain thresholds among individual animals. Throughout the study,
experimental group sizes increased from an initial sample size of 10 mice per group to a
sample size of 15 mice per group. The narrow range of baseline tail-flick latencies (2-3
seconds) resulted in exclusion of mice from each experimental group, thus requiring an
increase in sample size to 15 mice per experimental group. Therefore, data analysis of experimental groups with less than 10 mice per group, or less than 15 mice per group, occurred as a result of exclusion of mice with baseline tail-flick latencies outside the required 2-3 second range.

Figure 1. Ugo Basile tail-flick unit (Model 37360). The black arrow indicates the heat source.

Experimental Procedures

The study was divided into six separate experiments. The goal of experiment 1 was to determine whether atipamezole alone would affect tail-flick latencies. Mice were randomly divided into two groups: saline (n=10) and atipamezole (n=10). Mice were
injected with either 1 mg/kg atipamezole or saline (same volume) intraperitoneally (IP). Tail-flick latencies were measured at 15, 30 and 60 minutes following injection.

The purpose of experiment 2 was to evaluate tail-flick latencies of mice that received butorphanol following pretreatment with either atipamezole or saline. Mice were randomly divided into two groups: saline (n=10) and atipamezole (n=10). Mice were pretreated with either 1 mg/kg atipamezole or the same volume of saline IP. Approximately 10 minutes after treatment, both groups of mice were administered 1 mg/kg butorphanol IP. Tail-flick latencies were measured at 15, 30 and 60 minutes following butorphanol administration.

Experiment 3 was performed to evaluate the tail-flick latencies of mice that received low-dose buprenorphine following pretreatment with either atipamezole or saline. Mice were randomly divided into two groups: saline (n=11) and atipamezole (n=8). Mice were pretreated with either 1 mg/kg atipamezole or the same volume of saline IP. Approximately 10 minutes after treatment, both groups of mice received 0.05 mg/kg buprenorphine IP and tail-flick latencies were measured at 15, 30 and 60 minutes following injection with low-dose buprenorphine.

The goal of experiment 4 was to evaluate the antinociceptive properties of high-dose buprenorphine following pretreatment with atipamezole or saline. Mice were randomly divided into two groups: saline (n=9) and atipamezole (n=10). Mice were pretreated with either 1 mg/kg atipamezole or the same volume of saline IP. Approximately 10 minutes later, both groups of mice received 0.1 mg/kg buprenorphine IP. Tail-flick latencies were measured at 15, 30, 60, 90 and 120 minutes following high-dose buprenorphine administration.
To simulate a practical application of the study findings, mice were anesthetized as part of experiments 5 and 6. Experiment 5 was performed to evaluate the antinociceptive properties of butorphanol following ketamine-dexmedetomidine anesthesia with atipamezole reversal. Mice were randomly divided into three groups: atipamezole only (n=15), atipamezole plus butorphanol (n=15) and saline only (n=15). All three groups of mice were anesthetized with 50 mg/kg ketamine and 0.5 mg/kg dexmedetomidine IP. Mice were anesthetized at the same time of day, between 0900 and 1400 hours, to control for circadian variation. After induction of anesthesia, mice were placed in sternal recumbency on a 37°C water recirculating heating pad (Gaymar T/Pump® 500) for thermal support. Sterile ophthalmic ointment (Refresh Lacri-lube, Allergan, Inc.) was applied to the eyes to prevent corneal ulceration and eye trauma during anesthesia. The mice were assessed using the pedal withdrawal reflex every 1-2 minutes to determine if each animal reached a surgical plane of anesthesia. The time at which the pedal withdrawal reflex was lost was recorded. After a 30 minute period of anesthesia, mice received either 1 mg/kg atipamezole or the same volume of saline IP. Immediately after the mice regained their righting reflex, 1 mg/kg of butorphanol was administered IP to the appropriate group. Tail-flick latencies were then measured at 15, 30, 60, 90, 120 and 150 minutes following treatment with butorphanol.

The following time points were recorded:

- T0 = when ketamine-dexmedetomidine was administered
- T1 = when righting reflex was lost (point at which the mouse was unable to right itself after gently being tipped over)
- T2 = when atipamezole or saline was administered
- T3 = when righting reflex reappeared (point at which the mouse was able to right itself after gently being tipped over)

The goal of experiment 6 was to evaluate the antinociceptive properties of high dose buprenorphine following ketamine-dexmedetomidine anesthesia with atipamezole reversal. Mice were randomly divided into three groups: atipamezole only (n=15), atipamezole plus buprenorphine (n=15) and saline only (n=15). Mice were anesthetized and provided with supportive care as described for experiment 5. Similar to experiment 5, the time at which the pedal withdrawal reflex was lost was recorded. After a 30 minute period of anesthesia, mice received either 1 mg/kg atipamezole or the same volume of saline IP. After the mice regained their righting reflex, 0.1 mg/kg of buprenorphine was administered IP to the appropriate group. Tail-flick latencies were then measured at 15, 30, 60, 90, 120 and 150 minutes following treatment with buprenorphine. Time points T1-T3 were recorded as described for experiment 5.
Figure 2. Timeline illustrating procedures performed in experiments 5 and 6. “K-D” designates administration of ketamine and dexmedetomidine anesthesia. “Ax” represents a 30 minute period of anesthesia. “Reverse” indicates the administration of the α2-adrenoceptor antagonist, atipamezole. “Opioid” indicates the administration of either butorphanol (experiment 5) or buprenorphine (experiment 6). The tail-flick assay was performed at 15, 30, 60, 90, 120 and 150 minutes following treatment with butorphanol (experiment 5) or buprenorphine (experiment 6).

Statistical Analysis

Data was analyzed using GraphPad Prism version 5.02 statistical software (GraphPad Software Inc., San Diego, CA). All data were expressed as mean ± SD. A two-way analysis of variance (ANOVA) for repeated measures with time and treatment as the main factors was performed to analyze the data from experiments 1-4. Differences among treatments on tail-flick latencies at 15, 30, 60, 90, 120 and 150 minutes after drug administration were analyzed by two-way repeated measures ANOVA for data from experiments 5 and 6 followed by the Bonferroni multiple comparison test to determine which treatments at which time points differed. A value of $p < 0.05$ was considered statistically significant.
Chapter 3

Results

Effects of Atipamezole on Tail-Flick Latency

Tail-flick latencies in mice were analyzed at specific time points after IP administration of either 1 mg/kg atipamezole or saline. No significant differences in tail-flick latency between the atipamezole group and the control group were detected at 15, 30 and 60 minutes following administration ($p = 0.2951$, Figure 3).

![Experiment 1](image)

**Figure 3.** Effects of atipamezole alone on tail-flick latency. Bars indicate the mean ± SD for each of the groups: saline (n=10) and atipamezole (n=10). Baseline tail-flick latency for mice in Experiment 1 was 2.6 ± 0.2 sec.
Effects of Atipamezole on Antinociceptive Effects of Butorphanol

Mice were pretreated with an IP injection of either 1 mg/kg atipamezole or the same volume of saline. Approximately 10 minutes later, all mice were administered 1 mg/kg butorphanol IP. No significant differences in tail-flick latencies were detected between the atipamezole and butorphanol group versus the saline and butorphanol group at 15, 30 and 60 minutes following butorphanol treatment ($p = 0.7872$, Figure 4).

![Experiment 2 graph]

**Figure 4.** Effects of atipamezole plus butorphanol on tail-flick latency. Bars indicate the mean ± SD for each of the groups: atipamezole and butorphanol (n=10) and saline and butorphanol (n=10). Baseline tail-flick latency for mice in Experiment 2 was 2.5 ± 0.3 sec.
Effects of Atipamezole on Antinociceptive Effects of Low Dose Buprenorphine

In a similar manner, mice were pretreated with an IP injection of either 1 mg/kg atipamezole or the same volume of saline. Approximately 10 minutes later, all mice were administered 0.05 mg/kg buprenorphine IP. No significant differences in tail-flick latencies were detected between the atipamezole and buprenorphine group versus the saline and buprenorphine group at 15, 30 and 60 minutes following buprenorphine treatment \( (p = 0.6106, \text{ Figure 5}) \). There was a significant difference between time points \( (p < 0.0001) \), as tail-flick latencies increased in both groups from 15 minutes to 60 minutes post-buprenorphine administration.
Figure 5. Effects of atipamezole plus low dose (0.05 mg/kg) buprenorphine on tail-flick latency. Bars indicate the mean ± SD for each of the groups: atipamezole and low dose buprenorphine (n=8) and saline and low dose buprenorphine (n=11). Baseline tail-flick latency for mice in Experiment 3 was 2.4 ± 0.2 sec. There was a significant difference between time points (p < 0.0001), as tail-flick latencies increased in both groups from 15 minutes to 60 minutes post-buprenorphine administration.

Effects of Atipamezole on Antinociceptive Effects of High Dose Buprenorphine

Approximately 10 minutes after mice were pretreated with 1 mg/kg atipamezole or saline IP, all mice were administered 0.1 mg/kg buprenorphine IP. No significant differences in tail-flick latencies were detected between the atipamezole and buprenorphine group versus the saline and buprenorphine group at 15, 30, 60, 90 and 120 minutes following buprenorphine treatment (p = 0.8253, Figure 6). There was a
significant difference between time points \( p < 0.0001 \), as tail-flick latencies increased in both groups from 15 minutes to 120 minutes following administration of high dose buprenorphine.

Figure 6. Effects of atipamezole plus high dose (0.1 mg/kg) buprenorphine on tail-flick latency. Bars indicate the mean ± SD for each of the groups: atipamezole and high dose buprenorphine \( (n=10) \) and saline and high dose buprenorphine \( (n=9) \). Baseline tail-flick latency for mice in Experiment 4 was 2.6 ± 0.3 sec. There was a significant difference between time points \( p < 0.0001 \), as tail-flick latencies increased in both groups from 15 minutes to 120 minutes following administration of high dose buprenorphine.
Effects of Atipamezole on Antinociceptive Effects of Butorphanol Following Ketamine-Dexmedetomidine Anesthesia

After recovery from ketamine-dexmedetomidine anesthesia, there was no significant difference between the atipamezole and atipamezole plus butorphanol groups at all time points measured: 15, 30, 60, 90, 120 and 150 minutes after treatment ($p = 0.1572$, Figure 7). There were significantly higher tail-flick latency values in the ketamine-dexmedetomidine plus saline group than in the ketamine-dexmedetomidine plus atipamezole group at 30 and 60 minutes following treatment ($p < 0.001$).

Furthermore, there were significantly higher tail-flick latencies in the ketamine-dexmedetomidine plus saline group than in the ketamine-dexmedetomidine plus atipamezole and butorphanol group at 30, 60 and 120 minutes following treatment ($p < 0.05$, $p < 0.001$ and $p < 0.05$, respectively). There was a significant difference in comparing time following treatment ($p < 0.0001$), as tail-flick latencies increased in all groups from 15 minutes to 150 minutes following treatment.
Figure 7. Effects of atipamezole plus butorphanol on tail-flick latency following ketamine-dexmedetomidine anesthesia. Bars indicate the mean ± SD for each of the groups: ketamine-dexmedetomidine followed by atipamezole (n=15), ketamine-dexmedetomidine followed by atipamezole and butorphanol (n=15) and ketamine-dexmedetomidine followed by saline (n=15). Baseline tail-flick latency for mice in Experiment 5 was 2.6 ± 0.3 sec. There was a significant difference in comparing time following treatment (p < 0.0001), as tail-flick latencies increased in all groups from 15 minutes to 150 minutes following treatment.
Effects of Atipamezole on Antinociceptive Effects of High Dose Buprenorphine Following Ketamine-Dexmedetomidine Anesthesia

After recovery from ketamine-dexmedetomidine anesthesia, there was no significant difference between the atipamezole and atipamezole plus 0.1 mg/kg buprenorphine groups, except at 90 and 150 minutes after treatment ($p < 0.001$ and $p < 0.01$, respectively, Figure 8). There were significantly higher tail-flick latency values in the ketamine-dexmedetomidine plus saline group than in the ketamine-dexmedetomidine plus atipamezole group at 15, 30, 60, 90 and 120 minutes following treatment ($p < 0.01$, $p < 0.001$, $p < 0.001$ and $p < 0.01$, respectively). There was no significant difference between these two groups at 150 minutes ($p > 0.05$). Furthermore, there were no significant differences in tail-flick latencies in comparing the ketamine-dexmedetomidine plus saline group to the ketamine-dexmedetomidine plus atipamezole and 0.1 mg/kg buprenorphine group, except at 60 minutes following treatment ($p < 0.05$). There was a significant difference in comparing time following treatment ($p < 0.0001$), as tail-flick latencies increased in all groups from 15 minutes to 150 minutes following treatment.
Figure 8. Effects of atipamezole plus high dose buprenorphine on tail-flick latency following ketamine-dexmedetomidine anesthesia. Bars indicate the mean ± SD for each of the groups: ketamine-dexmedetomidine followed by atipamezole (n=15), ketamine-dexmedetomidine followed by atipamezole and 0.1 mg/kg buprenorphine (n=15) and ketamine-dexmedetomidine followed by saline (n=15). Significant difference in tail-flick latency between the atipamezole and ketamine/dexmedetomidine group in comparison to the atipamezole, 0.1 mg/kg buprenorphine and ketamine/dexmedetomidine group (**p < 0.01; ***p < 0.0001). Baseline tail-flick latency for mice in Experiment 6 was 2.8 ± 0.3 sec. There was a significant difference in comparing time following treatment (p < 0.0001), as tail-flick latencies increased in all groups from 15 minutes to 150 minutes following treatment.
Chapter 4

Discussion

Pain management following experimental procedures is a fundamental aspect of refinement of experimental methods. According to the eighth edition of the *Guide for the Care and Use of Laboratory Animals*, “an integral component of veterinary medical care is prevention or alleviation of pain associated with procedural and surgical protocols.”12 The Guide also states that “the proper use of anesthetics and analgesics in research animals is an ethical and scientific imperative.”12 Insufficient concentrations of analgesics, or analgesia that is inadvertently attenuated by the administration of a reversal agent, may result in pain and stress in an animal during the postoperative period. Pain and stress may bias study outcome by causing delayed surgical recovery and/or high levels of circulating corticosteroids.6,13 Therefore, poor post-procedural analgesia can have significant impacts on regulatory compliance, animal welfare and experimental results.

The hypothesis of the current study was that atipamezole may inhibit the antinociceptive effects of butorphanol, but would not affect the analgesic properties of buprenorphine in C57BL/6J mice. The tail-flick assay was utilized to evaluate the antinociceptive effects of butorphanol and buprenorphine following pretreatment with atipamezole, and also following ketamine-dexmedetomidine anesthesia. Evaluation of atipamezole alone indicated no significant change in tail-flick latency in comparison to the control group. Atipamezole had no significant effect on the antinociceptive properties of butorphanol or buprenorphine, while evaluating their effects with and without the use
of ketamine and dexmedetomidine anesthesia. Furthermore, butorphanol had less analgesic effect than buprenorphine, as indicated by a greater increase in tail-flick latencies following treatment with buprenorphine in comparison to butorphanol.

Experiments 2 and 5 evaluated the effects of atipamezole on the antinociceptive properties of butorphanol without and with ketamine-dexmedetomidine anesthesia, respectively. Unanesthetized mice that received butorphanol did not have significantly higher tail-flick latencies compared to baseline. There were no significant differences in tail-flick latencies between the mice that received atipamezole and butorphanol versus those that received saline and butorphanol. These results indicate that atipamezole did not change the antinociceptive effects of butorphanol in unanesthetized mice. Furthermore, there were no significant differences in tail-flick latencies among the mice that received atipamezole versus those that received atipamezole and butorphanol following ketamine and dexmedetomidine anesthesia. These results indicate that butorphanol produced weak analgesic effects and atipamezole did not attenuate these effects in both anesthetized and unanesthetized C57BL/6J mice.

Experiments 3, 4 and 6 investigated the effects of atipamezole on the antinociceptive properties of buprenorphine without and with anesthesia. There were no significant differences in tail-flick latencies between the mice that received atipamezole and buprenorphine versus those that received saline and buprenorphine. These results indicate that atipamezole did not change the antinociceptive effects of buprenorphine in unanesthetized mice. The results also show that high dose buprenorphine reached maximal effect at 90 minutes following treatment in unanesthetized mice.
Moreover, mice that received atipamezole and buprenorphine had significantly greater tail-flick latencies at 90 and 150 minutes following buprenorphine treatment, in comparison to the mice that received solely atipamezole following ketamine and dexmedetomidine anesthesia. Although the difference in tail-flick latencies was not statistically significant, there was a strong trend towards significance between the mice that received atipamezole and buprenorphine versus those that received only atipamezole following anesthesia at 120 minutes following treatment. These results demonstrate that atipamezole did not attenuate the analgesic effects of buprenorphine in neither anesthetized nor awake mice. Additionally, buprenorphine produced moderate analgesic effects in mice beginning at 90 minutes following treatment. Because the analgesic effects of buprenorphine were not evident until 90 minutes post-administration, we postulate that buprenorphine should be given prior to surgery to ensure adequate immediate postoperative analgesia.

Interestingly, experiments 5 and 6 displayed a return of analgesic effect after reversal of ketamine and dexmedetomidine anesthesia with atipamezole. In experiment 5, this finding is evidenced by increased tail-flick latencies at the 90, 120 and 150 minute time points in the K-D, atipamezole group; while increased tail-flick latencies at the 120 and 150 minute time points in the K-D, atipamezole group were present in experiment 6. This increase in tail-flick latencies, without the addition of butorphanol- or buprenorphine-induced analgesia, may have been attributed to residual analgesic effects of ketamine. Because atipamezole competitively inhibits α2-adrenergic receptors, it is unlikely there was a return of analgesic effects from dexmedetomidine following reversal. Furthermore, the analgesic effect produced with buprenorphine treatment
following ketamine and dexmedetomidine anesthesia was likely due to an additive effect of buprenorphine and ketamine.

The results of the current study disagree with the findings of Jang et al., who investigated the effects of atipamezole on the antinociceptive properties of butorphanol in rats. Jang et al. concluded that atipamezole partly altered the antinociceptive effects of butorphanol in adult male Sprague-Dawley rats as measured by tail-withdrawal from a 50°C water bath. The authors postulated that atipamezole may inhibit the activity of serotonergic pathways by blocking α2-adrenoceptors. As a result, butorphanol antinociception was likely inhibited via the inactivation of serotonergic pathways, as the drug mainly acts on the κ-opioid receptor. The results of the current study suggest similar conclusions cannot be applied to mice, as the antinociceptive effects of butorphanol and buprenorphine were not affected by atipamezole. The discrepancy in results between the current study and those of Jang et al. may be due to a difference in distribution and pharmacology of serotonergic receptors in rats versus mice.

The findings of the current study support those reported in several previous studies that found butorphanol to provide weak analgesic effects in comparison to other opioids in mice. Gades et al. found butorphanol consistently provided the lowest magnitude of effect with the shortest duration of analgesia in comparison to buprenorphine and morphine in mice and rats. Garner et al. found less-than-maximal analgesia was obtained with the use of butorphanol in mice. Butorphanol analgesia in mice and rats only lasts 1-2 hours, requiring frequent dosing, which is often impractical in a laboratory setting. Similar to our results, Gades et al. concluded that buprenorphine produced an intermediate analgesic effect in mice using the tail-flick assay. Unlike
butorphanol, buprenorphine has a long duration of action, lasting 3-5 hours in mice and 6-8 hours in rats. Our study results further support this, as tail-flick latencies increased with time following buprenorphine treatment with and without anesthesia.

In addition to analgesic efficacy and duration of action, one must also evaluate the time until the onset of the analgesic effect occurs. Results from the present investigation showed that the onset of analgesia was not present until 90 minutes after buprenorphine administration. Understanding the onset of analgesic effect is crucial to determining when to administer the drug: preoperatively, intraoperatively, or immediately postoperatively. The results of this study suggest that buprenorphine should be administered either immediately preoperatively or intraoperatively, depending on the duration of the surgical procedure to be performed, in order to ensure that the mouse has sufficient analgesia on board immediately upon recovery from anesthesia.

Moreover, an investigator should consider potential adverse effects while selecting analgesics and anesthetics. Several observations of clinical relevance were made during analgesiometry testing. Intraperitoneal administration of butorphanol alone produced marked sedation in female C57BL/6J mice, as evidenced by reduced activity and decreased movement within the cage. Additionally, only 63% of mice anesthetized with ketamine and dexmedetomidine lost their pedal withdrawal reflex over a 30 minute period of anesthesia. In the mice that did lose the pedal withdrawal reflex, it took approximately 19 minutes following ketamine and dexmedetomidine administration. These results suggest that a combination of ketamine and dexmedetomidine produced inconsistent levels of anesthesia in female C57BL/6J mice.
Furthermore, the loss of righting reflex was between 1-2 minutes following injection with ketamine and dexmedetomidine. Following a 30-minute period of anesthesia, mice reversed with atipamezole regained their righting reflex approximately 10 minutes following administration of the reversal agent (Tables 1 and 3). In this study, it can be concluded that atipamezole reliably and effectively reversed ketamine and dexmedetomidine anesthesia in female C57BL/6J mice. The results of the current study agree with those of Cruz et al., which found the administration of atipamezole following ketamine and medetomidine anesthesia produced a more rapid recovery in Swiss Webster mice.4

Alternatively, mice that were not reversed from anesthesia recovered naturally approximately 3 hours following injection with ketamine and dexmedetomidine (Tables 2 and 4). Based on the findings in this study, an injectable anesthetic combination of ketamine and dexmedetomidine resulted in a rapid loss of righting reflex, but did not reliably produce a surgical plane of anesthesia in C57BL/6J mice. Moreover, the reversal of anesthesia with atipamezole reliably produced a rapid return of righting reflex in C57BL/6J mice following ketamine and dexmedetomidine anesthesia.

Further research is needed to characterize the optimal time to administer buprenorphine to obtain peak postoperative analgesia in C57BL/6J mice. The findings in this study would be more robust if they were supported by multiple analgesiometry tests. It would be interesting to see if the same results were collected using the hot-plate test as an alternative measurement of acute pain. Moreover, it is generally recommended that another person prepares and injects the analgesic agents in order to blind the individual who performs the tail-flick testing of the treatment.2 The individual who performed the
tail-flick assay in these experiments was not blinded, which is a limitation of the current study.

To our knowledge, this study is the first to evaluate the potential effects of the α2-adrenergic antagonist, atipamezole, on the antinociceptive properties of butorphanol and buprenorphine in mice. In conclusion, atipamezole administered to hasten recovery from anesthesia does not interfere with the postoperative analgesic effect of butorphanol or buprenorphine in mice. Based on the results of this study, it is suggested that buprenorphine be administered prior to surgery to provide sufficient postoperative analgesia in female C57BL/6J mice. The information gained from this study contributes to the development of optimal analgesic protocols to ensure the welfare of laboratory mice.
Chapter 5

Future Directions

Because the analgesic effects of buprenorphine were not evident until 90 minutes post-administration, it is proposed that buprenorphine should be given prior to surgery to ensure adequate immediate postoperative analgesia. To further explore this, I plan on using the tail-flick assay to evaluate the antinociceptive effects of buprenorphine administered concurrently with the induction of ketamine and dexmedetomidine anesthesia in female C57BL/6J mice. Two experimental groups are proposed: atipamezole plus ketamine/dexmedetomidine (n=15); and atipamezole plus 0.1 mg/kg buprenorphine and ketamine/dexmedetomidine (n=15). In order to improve accuracy, three separate measurements of baseline tail-flick latencies will be recorded per mouse. These numbers will be averaged to represent the baseline latency for each individual mouse. The measurement of baseline tail-flick latency is a modification of previous methods. This method of obtaining baseline tail-flick latencies reflects the basic protocol for measurement of nociception using the tail-flick assay and will likely result in a reduction of animal numbers required for the experiment. Nociception will be evaluated at 15, 30, 60, 90, 120 and 150 minutes following reversal with atipamezole. In this way, I can investigate if atipamezole affects the antinociceptive properties of preemptive buprenorphine, and evaluate whether the preemptive use of buprenorphine provides stronger analgesic effects to mice during the immediate postoperative recovery period. By measuring the time until the loss of righting reflex in addition to the presence or
absence of the pedal withdrawal reflex, I will be able to evaluate if the preemptive 
addition of buprenorphine has any synergistic effect on these parameters as well.
References


3. **Bennett GJ.** 2000. Update on the neurophysiology of pain transmission and modulation: focus on the NMDA receptor. J Pain Symptom Manage **19:**S2-S6.

4. **Cruz JI, Loste JM, Burzaco OH.** 1998. Observations on the use of medetomidine/ketamine and its reversal with atipamezole for chemical restraint in the mouse. Laboratory Animals **32:**18-22.


### Appendix A

#### Raw Data Experiments 5 and 6

Table 1. Experiment 5 - Mice anesthetized with ketamine-dexmedetomidine with atipamezole reversal. LORR – loss of righting reflex; RORR – return of righting reflex.

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Table 3. Experiment 6 - Mice anesthetized with ketamine-dexmedetomidine with atipamezole reversal. LORR – loss of righting reflex; RORR – return of righting reflex.

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<th>Time to Loss of Pedal Withdrawal Reflex following Anesthesia Induction (min)</th>
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Table 4. Experiment 6 – Mice anesthetized with ketamine-dexmedetomidine without atipamezole reversal. LORR – loss of righting reflex; RORR – return of righting reflex.

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Appendix B

Literature Review

Butorphanol

Butorphanol is an example of an opioid drug used in veterinary medicine that is a weak antagonist of \( \mu \) receptors and a relatively strong \( \kappa \) receptor agonist.\(^6\) Butorphanol is used in many species as an analgesic, premedication, antiemetic or antitussive. It has been FDA-approved for use in dogs for the relief of chronic, non-productive coughing originating from inflammatory conditions of the upper respiratory tract. In horses, it has been FDA-approved for the relief of pain associated with colic in adult horses and yearlings.\(^{19}\)

Analgesic efficacy of butorphanol has been reported to be less than that of morphine, oxymorphone, buprenorphine, keterolac or ketoprofen.\(^6\) In comparison to morphine and buprenorphine, butorphanol produced the lowest level and the shortest duration (1-2 hours) of analgesia in mice and rats.\(^7\) Gades et al. recommend the use of butorphanol for mild pain of short duration with a dosing interval of 1-2 hours in both rats and mice.\(^7\) Other studies performed by Pircio et al. and Garner et al. determined that butorphanol has relatively poor analgesic effects and morphine produces greater analgesia than butorphanol using conventional analgesia tests.\(^8,18\) Garner et al. found that administering butorphanol at the highest possible nonlethal dose to Swiss-Webster mice produced only partial analgesia.\(^8\) This is likely due to a ceiling effect that is reached at
higher dosages, where analgesia is no longer improved and alternatively, may actually become reduced.\textsuperscript{19}

Compared with other opioid analgesics, butorphanol is not very useful in both small animal species and lab animal species, such as mice and rats, for the treatment of severe pain. Furthermore, due to its short duration of action, the drug must be dosed frequently.\textsuperscript{19} As a pure $\mu$ antagonist, butorphanol can be administered to reverse the CNS and respiratory depressant effects of $\mu$ agonists. As a result of its effects on the $\kappa$ receptors, butorphanol can be used to reverse these effects without completely reversing the analgesic effect of the $\mu$ agonist drug.\textsuperscript{19} Interestingly, Morgan et al. investigated the effects of various opioids in different strains and stocks of rats by using a tail withdrawal and drug discrimination procedure.\textsuperscript{15} This study concluded that butorphanol produced maximal analgesic effects in F344 and Sprague-Dawley rats at low doses, half-maximal effects in Long-Evans rats and no analgesic effect in Lewis rats. Therefore, there were differences in analgesic effects of opioids across rat strains and stocks.\textsuperscript{15}

Butorphanol is metabolized by the liver. Metabolism of the drug occurs mainly by hydroxylation. The metabolites of butorphanol and the parent compound are excreted primarily into urine, with only 5\% excreted unchanged. Approximately 11-14\% of a dose of butorphanol is excreted into bile and eliminated within the feces.\textsuperscript{19} Adverse effects of the drug reported in dogs and cats include respiratory depression, ataxia, sedation, excitement and rarely anorexia or diarrhea. Sedation was an adverse effect noted with butorphanol administration in female C57BL/6J in my study. In general, adverse effects of butorphanol are typically less severe than those seen with pure $\mu$ agonists. For instance, butorphanol administration may produce increased parasympathetic tone,
resulting in mild bradycardia and mild decreases in arterial blood pressures. However, the cardiovascular effects of butorphanol are much less than pure μ agonists. Butorphanol is a class IV controlled substance with a minimal risk of causing physical dependence in veterinary species.  

Buprenorphine

Buprenorphine has partial agonist activity at the μ receptor. Buprenorphine is considered 25 times as potent as morphine when administered intramuscularly and produces dose-related analgesia. Similar to butorphanol, buprenorphine has a ceiling effect, which causes analgesic effects to be reduced at higher doses. The maximum analgesic effect of buprenorphine is less than that of morphine, but greater than that of butorphanol. Analgesiometric assays performed by Gades et al. determined that buprenorphine consistently provided an intermediate analgesic effect and had the longest duration of action in comparison to butorphanol and morphine in mice and rats. Its duration of action lasted approximately 6-8 hours in rats and 3-5 hours in mice. Hot plate and tail-flick evaluations by Gades et al. determined that like butorphanol, buprenorphine is not an effective analgesic for severe pain. Other studies have not shown any significant differences between buprenorphine and various NSAIDs in rats following abdominal surgery.

Buprenorphine is most commonly used as an analgesic for mild to moderate pain in dogs and cats. In cats, the drug is often administered by the oral transmucosal route for control of post-operative pain. This partial μ agonist has a high affinity for μ receptors.
in the CNS, likely contributing to its long duration of action. Because it has a relatively long duration of action and due to the extensive data available on its dosage and efficacy, buprenorphine is commonly used in laboratory animal medicine. Similar to butorphanol, it has a lower abuse potential than pure μ opioid agonists, such as morphine and fentanyl.

Although buprenorphine is not as effective an analgesic as pure μ agonists in many species, it generally results in fewer adverse effects. Cardiovascular effects include mild hypotension and bradycardia. Respiratory depression may occur with buprenorphine administration. Additionally, sedation has been reported in various species. Administration of buprenorphine may result in increased motor activity, decreased intestinal activity and the drug has been associated with pica in rats. Buprenorphine may cause urine retention or difficulty voiding. No adverse effects were observed in female C57BL/6J mice following treatment with buprenorphine in my current study.

Metabolism of buprenorphine occurs in the liver by N-dealkylation and glucuronidation. Approximately 70% of the metabolites of the drug are eliminated by biliary excretion into the feces, while approximately 27% of metabolites are excreted in the urine. In rats, considerable enterohepatic recirculation occurs. Ohtani et al. reported the serum half-life of buprenorphine to be 2.8 hours in rats. Because buprenorphine is a controlled substance, a drug enforcement administration (DEA) license is required prior to use of this analgesic.
Dexmedetomidine

Dexmedetomidine is the dextrorotatory enantiomer of the alpha-2 adrenergic agonist, medetomidine. Medetomidine is a racemic mixture of dexmedetomidine and levomedetomidine. Levomedetomidine is pharmacologically inactive, so dexmedetomidine is approximately 2 times more potent than medetomidine. In comparison to xylazine, dexmedetomidine is much more specific for alpha-2 receptors versus alpha-1 receptors. The α2-adrenergic receptors are located in various tissues throughout the body, both presynaptically and postsynaptically in neuronal and non-neuronal tissues. Moreover, α2-adrenergic receptors can be found extrasynaptically in the vasculature as well.

Dexmedetomidine has been FDA-approved for use as a sedative and analgesic in cats and dogs. In the United States, the drug is indicated for use as a pre-anesthetic to general anesthesia in dogs only; however, dexmedetomidine is indicated for use as a premedication prior to ketamine general anesthesia in cats in European countries. The α2-adrenergic agonists are commonly used in veterinary medicine for their consistent anxiolysis, sedation, analgesia and muscle relaxation. Furthermore, α2-agonists administered at low doses significantly reduce the amount of injectable or inhalant agent required to induce and maintain general anesthesia. The anesthetic sparing effect nearly correlates with the affinity of the drugs for the α2-adrenergic receptors. Sinclair states the more specific the α2-agonist, the greater the anesthetic sparing effect.

The adverse effects reported with medetomidine or dexmedetomidine include bradycardia, vasoconstriction, muscle tremors, transient hypertension, reduced tear
production, 1st and 2nd degree atrioventricular blocks, respiratory depression, hypothermia, urination, vomiting, hyperglycemia and pain upon intramuscular injection. The hemodynamic effects of α2-adrenergic agonists in dogs have been described as a biphasic blood pressure response. During phase 1, activation of postsynaptic α2-receptors in peripheral vascular smooth muscle results in peripheral vasoconstriction with a subsequent increase in blood pressure. In phase 2, vasoconstriction decreases, resulting in a decrease in blood pressure. During this phase, a central hypotensive effect predominates, sympathetic nervous tone is decreased and prolonged bradycardia develops. Although the exact mechanism is unknown, cardiac output also decreases following α2-agonist administration in dogs. Pypendop et al. found that cardiac output can be dramatically reduced by up to 50% (L blood/min) following medetomidine administration in dogs. Rare adverse effects that have been reported include prolonged sedation, paradoxical excitation, hypersensitivity, pulmonary edema, apnea and death from circulatory failure.

Following intramuscular administration in dogs, dexmedetomidine reaches peak plasma levels in approximately 35 minutes. In cats, the drug reaches peak plasma levels in about 15 minutes following intramuscular administration. The drug is predominantly metabolized in the liver via glucuronidation and N-methylation. Metabolites of dexmedetomidine are eliminated primarily in the urine and to a lesser extent in the feces. The elimination half-life is approximately 40-50 minutes in dogs, whereas it is equal to approximately 1 hour in cats. Molina and Herrero found the effects of medetomidine on allodynia and hyperalgesia in rats were significantly reduced within 1 hour of administration.
Atipamezole

Atipamezole is an imidazole α2-adrenergic antagonist that competitively inhibits α2-adrenergic receptors. As a result, atipamezole acts as a reversal agent for α2-adrenergic agonists, including medetomidine and dexmedetomidine. Atipamezole could potentially be used for reversal of other α2-agonists as well, such as clonidine and xylazine. Pharmacologic effects of the drug include a reduction of sedation, a decrease in blood pressure, increased respiratory and heart rates and reduction of the analgesic effects of α2-adrenergic agonists. In small mammals such as mice and rats, atipamezole has the advantage of reducing the risk of hypothermia by hastening recovery from anesthesia. Furthermore, the competitive antagonism of α2-adrenergic antagonists is critically important in reversing potentially life-threatening cardiovascular effects or in the case of an inadvertent α2-agonist overdose. Because atipamezole effectively reverses the analgesic effects of α2-adrenergic agonists, additional analgesia should be considered, especially following painful or invasive procedures.

Atipamezole is licensed for intramuscular use; however, in emergency situations, intravenous administration may be appropriate. Sudden fluctuations in cardiovascular function have been reported to occur following intravenous administration. Consequently, intramuscular administration of the drug is recommended to facilitate a gradual awakening and to minimize changes in blood pressure, heart rate and cardiac output.

Potential adverse effects of atipamezole administration include occasional vomiting, diarrhea, tremors, hypersalivation and brief excitation or apprehensiveness.
Additional adverse effects of α2-adrenergic antagonists include tachycardia and hypotension.\textsuperscript{24} A transient decrease in blood pressure has been reported to occur even with intramuscular administration of atipamezole in medetomidine-sedated dogs.\textsuperscript{28} A study performed by Kauppila et al. found that atipamezole dosed at 1.5 mg/kg IP decreased TFL in comparison to baseline latencies in rats, suggesting a hyperalgesic effect of the drug.\textsuperscript{12} Atipamezole administered alone in comparison to a saline control group did not decrease (nor increase) tail-flick latencies in female C57BL/6J mice in the current study. There were no significant differences in tail-flick latencies among these two groups of mice using the tail-flick assay.

**Combination of Ketamine and Dexmedetomidine**

A major advantage to the use of a combination of ketamine and dexmedetomidine is the ability to reverse sedation with atipamezole. Furthermore, in combination with ketamine, medetomidine or dexmedetomidine can provide muscle relaxation and additional analgesia. A combination of ketamine and medetomidine provides chemical restraint for minor procedures in mice, such as retroorbital bleeding, with rapid reversal by atipamezole.\textsuperscript{4,26} Cruz et al. and Taylor et al. also determined that female mice tend to be more resistant to the effects of the anesthetic combination of ketamine and medetomidine compared with male mice.\textsuperscript{4,26} Hahn et al. found the combination of ketamine and medetomidine used in rodents in field studies induced anesthesia within 1 minute.\textsuperscript{11} Additionally, less muscle tissue inflammation has been observed when
medetomidine is combined with ketamine versus the use of ketamine alone for intramuscular injections in rats.\(^2\) Due to a low pH of ketamine, intramuscular injection of the drug may cause discomfort and tissue reactions in small rodents.\(^2\) Therefore, a lesser concentration of ketamine is used in multimodal treatment with medetomidine, likely contributing to less tissue inflammation.\(^2\) As dexmedetomidine is a relatively new drug used within the field of veterinary medicine, limited published literature is currently available pertaining to the combination of ketamine and dexmedetomidine in laboratory animal species. In the current study, this anesthetic combination produced a rapid loss of righting reflex, but did not reliably produce a surgical plane of anesthesia in female C57BL/6J mice.

**Tail-Flick Assay**

Experimental models of nociception include tests that evaluate acute pain as well as persistent pain models. Two commonly used methods to model acute thermal pain include the hot-plate and tail-flick tests.\(^1\) The tail-flick test is a commonly used assay to measure a spinal reflex in response to thermal pain. The method was first described by D’Amour and Smith in 1941. The tail-flick unit accurately measures the nociceptive threshold in response to an infrared heat stimulus on the tail through the use of an adjustable heat stimulus. In addition to being commercially available, other advantages of the tail-flick assay include high reproducibility of test results, automatic detection of
animal response, and the ability to program a thermal stimulus cut-off time to avoid tissue damage.

According to Bannon et al., animals should be acclimated within the test room for a period of 30 minutes prior to the start of tail-flick testing.\(^1\) Separate groups of animals should be used for each treatment and baseline tail-flick latencies should be measured prior to any drug treatment. Mogil et al. found that gentle restraint of the animal using a cloth or cardboard holder creates less stress-induced analgesia in comparison to restraint with a Plexiglas cylinder.\(^13\) This is important because restraint stress has been reported to significantly modify both behavioral and physiological responses to pain, including stress-induced analgesia.\(^20\) In addition to restraint, other factors that may influence tail-flick latencies include drugs that affect muscle tone as well as tail temperature.\(^1\) The heat stimulus should be focused ~15 mm from the tip of the distal tail for mice. If the mouse does not produce a withdrawal reflex, a cutoff time of 10 seconds should be used to avoid tissue damage.\(^1\)

The test and control agents should be prepared in a way that they are less than 10 ml/kg for mice. In a randomized order, mice should be injected using a 1 ml syringe and a 30 gauge needle. It is crucial to stagger the injections to allow the operator to have enough time to perform the tail-flick assay at the same time interval after treatment of each mouse. The tail-flick test can then be performed at preferred time points following injection with the test or control agent. For example, tail-flick testing may be performed at 15, 30 and 60 minutes after injection of the agent. The tail-flick latency, or the time required for the mouse to show a tail-flick response, should then be recorded. A value of
10 seconds, equivalent to the cutoff time, should be recorded if no tail-flick response is observed.¹

Lastly, it is important to perform only one post-drug tail-flick assay at each time point, as repeated exposure of the tail may result in tissue damage. A sample size of 8 mice per group is sufficient for statistical analysis of multiple groups using ANOVA followed by a post-hoc test.¹ Because the noxious stimulus is transient and escapable in the tail-flick assay, the use of this test typically creates no serious ethical concerns.⁶
References


