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Atipamezole Reverses Ketamine–Dexmedetomidine Anesthesia without Altering the Antinociceptive Effects of Butorphanol and Buprenorphine in Female C57BL/6J Mice

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Butorphanol and buprenorphine are common analgesics used in laboratory mice. Inadvertent attenuation of the antinociceptive effects of these analgesics via the administration of an anesthetic reversal agent could result in postprocedural pain and distress, with subsequent negative effects on animal welfare, study outcomes, and regulatory compliance. This study was undertaken to determine whether atipamezole reverses ketamine–dexmedetomidine anesthesia and alters the antinociceptive effects of butorphanol and buprenorphine in female C57BL/6J mice. Atipamezole reliably reversed the anesthetic effects of ketamine–dexmedetomidine, and mice were ambulatory 17.4 ± 30.6 min after administration of the α_2-adrenoreceptor antagonist. Atipamezole alone had no significant effect on tail-flick latency and did not alter the antinociceptive properties of butorphanol or low-dose (0.05 mg/kg) or high-dose (0.1 mg/kg) buprenorphine in female C57BL/6J mice. After reversal of ketamine–dexmedetomidine anesthesia, tail-flick latency at 30, 60, and 150 min after analgesic treatment differed significantly between mice treated with atipamezole alone and those given atipamezole followed by butorphanol or high-dose buprenorphine. These results suggest that the analgesic effects of butorphanol and buprenorphine are not affected by atipamezole. Buprenorphine (0.1 mg/kg) administered 30 min prior to or at the time of anesthesia resulted in a greater magnitude of antinociception after antagonism of anesthesia than when given at the time of reversal. Given these results, we recommend the use of ketamine–dexmedetomidine anesthesia with buprenorphine administered either preemptively or at the time of anesthetic induction to provide a defined period of surgical anesthesia that is effectively reversed by atipamezole.

Abbreviations: MPE, maximal possible effect; TFL, tail-flick latency.

The ability to reverse anesthesia when a procedure is finished and avoid the complications of prolonged anesthesia, most notably hypothermia and dehydration, is highly desirable. Anesthetic reversal is advantageous in mice and rats by hastening recovery to reduce the risk of hypothermia, which is a considerable problem associated with anesthesia in these species.[9,14,18] The availability of the highly selective α_2 adrenoreceptor agonists medetomidine and dexmedetomidine and the specific α_2 adrenoreceptor antagonist atipamezole has renewed interest in the capabilities of reversing the anesthetic state when these agonists are used in combination with ketamine.[1,9,19,28] Atipamezole is a highly selective α_2 adrenoceptor antagonist that is used to decrease recovery time after the administration of the α_2 adrenoceptor agonists medetomidine and dexmedetomidine.[9,31] Dexmedetomidine is the active isomer of medetomidine and binds to α_2 adrenergic receptors with high specificity. For general anesthesia of rodents, α_2 adrenoceptor agonists frequently are combined with ketamine to provide muscle relaxation, enhance overall analgesia, and smooth recovery from ketamine anesthesia. However, α_2 adrenoceptor agonists have several undesirable side effects, including bradycardia, hypotension, respiratory depression, and hypothermia.[1,9,31,41] Atipamezole reverses the effects of the α_2 adrenoceptor agonists on central and peripheral receptors to restore cardiovascular and respiratory function.[24]

Although atipamezole reverses the cardiovascular and sedative effects of α_2 adrenoceptor agonists, it also reverses the analgesic effects of these drugs.[1,25] As a result, it is common for other analgesics, frequently opioids, to be administered postoperatively to prevent pain associated with various surgical procedures. Previous studies have shown the attenuation of analgesic effects of opioids after the administration of various anesthetic reversal agents.[6,22,25] Studies have found that systemic yohimbine in rats blocks the analgesic effect of systemic morphine in the tail-flick test.[6,22] Another investigation demonstrated that atipamezole attenuated the analgesic effects of butorphanol in Sprague–Dawley rats,[12] but the tail-flick latency (TFL) of the rats was not measured after the administration of atipamezole alone, allowing a potentially unidentified hyperalgesic effect of the α_2 adrenoceptor antagonist.[25] To our knowledge, no studies have examined the potential effects of atipamezole on butorphanol and buprenorphine in female C57BL/6J mice. Furthermore, a previous study concluded that atipamezole reverses the effects of ketamine and medetomidine anesthesia in male and female Swiss Webster mice,[9] but no studies have evaluated the efficacy of atipamezole in the reversal of ketamine and dexmedetomidine in female C57BL/6J mice.

The present investigation was undertaken to assess whether atipamezole reverses ketamine–dexmedetomidine (KD) anesthesia in female C57BL/6J mice and how atipamezole might affect

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opioid-induced thermal antinociception. In addition, butorphanol and buprenorphine were evaluated for potentiation of antinociception in the presence of atipamezole with or without prior KD anesthesia. Because attenuated analgesia could have substantial consequences on animal welfare, regulatory compliance, and scientific results in one of the primary species used in animal-based research, this area of investigation is important. Moreover, the present study is worthwhile because the recent introduction of dexmedetomidine into the US market has largely resulted in the substitution of this enantiomer for the racemic medetomidine as the α_2 adrenoceptor agonist of choice in veterinary practice. To our knowledge, antinociception after the reversal of KD anesthesia in mice has not previously been evaluated rigorously.

Materials and Methods

Animals. Female C57BL/6J mice (*Mus musculus*, *n* = 207; age, 3 to 4 wk) were obtained from Jackson Laboratories (Bar Harbor, ME). 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*.[23] Mice were maintained free of mouse hepatitis virus, minute virus of mice, mouse parvovirus, mouse norovirus, Theiler murine encephalomyelitis virus, mouse rotavirus, Sendai virus, pneumonia virus of mice, reovirus 3, lymphocytic choriomeningitis virus, ectromelia virus, mouse adenovirus 1, mouse adenovirus 2, polyoma virus, mouse cytomegalovirus, *Encephalitozoon cuniculi*, cilia-associated respiratory bacillus, *Clostridium piliforme*, *Mycoplasma pulmonis*, and endo- and ectoparasites. Mice were housed 5 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 nesting pads (Nestlets, Ancare, Bellmore, NY) for environmental enrichment. Mice received ad libitum standard commercial rodent chow (Teklad 2018 Global 18% Protein Rodent Diet, Harlan) and municipal tap water provided in bottles. The animal housing room was maintained at 21 ± 0.5 °C, 30% to 70% relative humidity, and 18 air changes hourly and on a 12:12-h light:dark cycle (lights on, 0700; lights off, 1900; no twilight). Mice underwent an acclimation period of 5 d before studies were initiated. All procedures were approved by the IACUC of the Pennsylvania State University College of Medicine.

Drugs. Atipamezole hydrochloride (1 mg/kg; Antisedan, Pfizer Animal Health, New York, NY), butorphanol tartrate (1 mg/kg; Torbugesic, Fort Dodge Animal Health, Fort Dodge, IA), and buprenorphine hydrochloride (0.05 to 0.1 mg/kg; Buprenex, Reckitt Benckiser Pharmaceuticals, Richmond, VA) were diluted with sterile water for more accurate dosing of the drugs. The final concentrations of the diluted drugs were: atipamezole, 0.25 mg/mL; butorphanol, 0.5 mg/mL; and buprenorphine, 0.015 mg/mL. At these concentrations, all drugs were administered in approximately equivalent volumes of 0.05 to 0.1 mL per animal. A subgroup of mice was anesthetized with ketamine hydrochloride (50 mg/kg; Ketathesia, Butler Animal Health Supply, Dublin, OH) and dexmedetomidine hydrochloride (0.5 mg/kg; Dexdomitor, Pfizer Animal Health). The anesthetic mixture was prepared by combining the drugs, diluting the mixture with sterile isotonic saline, and subsequently dosing at 0.2 mL/10 g to allow delivery of 50 mg ketamine and 0.5 mg dexmedetomidine in 0.2 mL. After measurement of baseline TFL, all drugs were administered by the intraperitoneal route. To avoid the confounding influence of insufficient drug washout, each naïve mouse experienced only a single treatment condition and was euthanized at the end of the study. Therefore, each mouse was restricted to a single study cohort.

Assessment of antinociception. Analgesiometric testing was performed in a dedicated procedure room that was separate from the animal housing room. The tail-flick test (model 37360, Ugo Basile, Schwenksville, PA) was used to assess nociception by measuring the nociceptive threshold to an infrared heat source on the tail. The tail-flick test was used to determine antinociception in this study because it 1) is a sensitive, quantifiable, and repeatable measure of reflex pain that highly correlates with the analgesic properties of drugs used in humans; 2) it selectively stimulates thermal nociceptors, unlike mechanical stimuli, which activate both mechanonociceptors and mechanoceptors; and 3) is independent of drug-induced changes in motor function.[12,26,38] The cutoff time of the infrared radiant heat source (setting, 60) was 10 s, to prevent tissue injury to the tail.[2] All experiments were performed at the same time of day, between 0900 and 1400 h, to control for circadian variation. To avoid a stress response on the test day, mice were acclimated to gentle restraint on the tail-flick unit for 3 d prior to testing. Each mouse was lightly restrained for 20 to 30 s by using a disposable blue underpad. Mice were habituated for 30 min in the procedure room on the day of testing. Each mouse was weighed, and the dorsal aspect of their tails was marked with a nontoxic, 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, to minimize the tail skin becoming sensitized or desensitized to the heat stimulus. Nonpigmented regions of the tail were avoided during tail-flick testing, given that nonpigmented regions of the C57BL/6J mouse tail show significantly increased response latency in the tail-flick assay.[45] Baseline TFL was measured at the first (most distal) increment on the tail. In accordance with previous studies using the tail-flick assay, only mice with baseline TFL between 2 and 3 s were included in the study.[15-17] Of the 289 mice evaluated, 82 mice had baseline latencies that fell outside this range and therefore were excluded from further analysis, resulting in a study population of 207 female C57BL/6J mice.

Experimental design. The effects of atipamezole on the antinociceptive properties of butorphanol and buprenorphine were evaluated. Naïve mice were assigned to 1 of 4 treatment conditions: atipamezole alone (*n* = 8); atipamezole followed by butorphanol (*n* = 9); atipamezole followed by low-dose (0.05 mg/kg) buprenorphine (*n* = 8); and atipamezole followed by high-dose (0.1 mg/kg) buprenorphine (*n* = 10). Because buprenorphine is the analgesic used most frequently in rodents undergoing surgical procedures, we chose to evaluate 2 commonly administered dosages of the drug.[4,42] A vehicle (saline) control group was used for each of these experimental groups (total, *n* = 37). Butorphanol or buprenorphine was administered approximately 10 min after treatment with atipamezole. In accordance with standard protocol for the tail-flick assay, TFL was measured at 15, 30, 60, 90, and 120 min after analgesic drug treatment.[2]

Next, antinociception was measured in naïve mice after recovery from KD anesthesia. After the induction of anesthesia, mice were placed in sternal recumbency on a 37 °C water recirculating heating pad (T/Pump 500, Gaymar Industries, Orchard Park, NY) for thermal support. The pedal withdrawal reflex was assessed via a gentle pinch of the toes of both hindlimbs every 1 to 2 min to determine whether each animal had reached a surgical plane of anesthesia. The presence or absence of the withdraw reflex, and if absent, the time at which the pedal withdrawal reflex was lost, was recorded. After a 30-min period of anesthesia, mice received either atipamezole or an equal volume of saline (Figure 1). Atipamezole was administered after a 30-min period of anesthesia, and TFL was measured at 15, 30, 60, 90, and 120

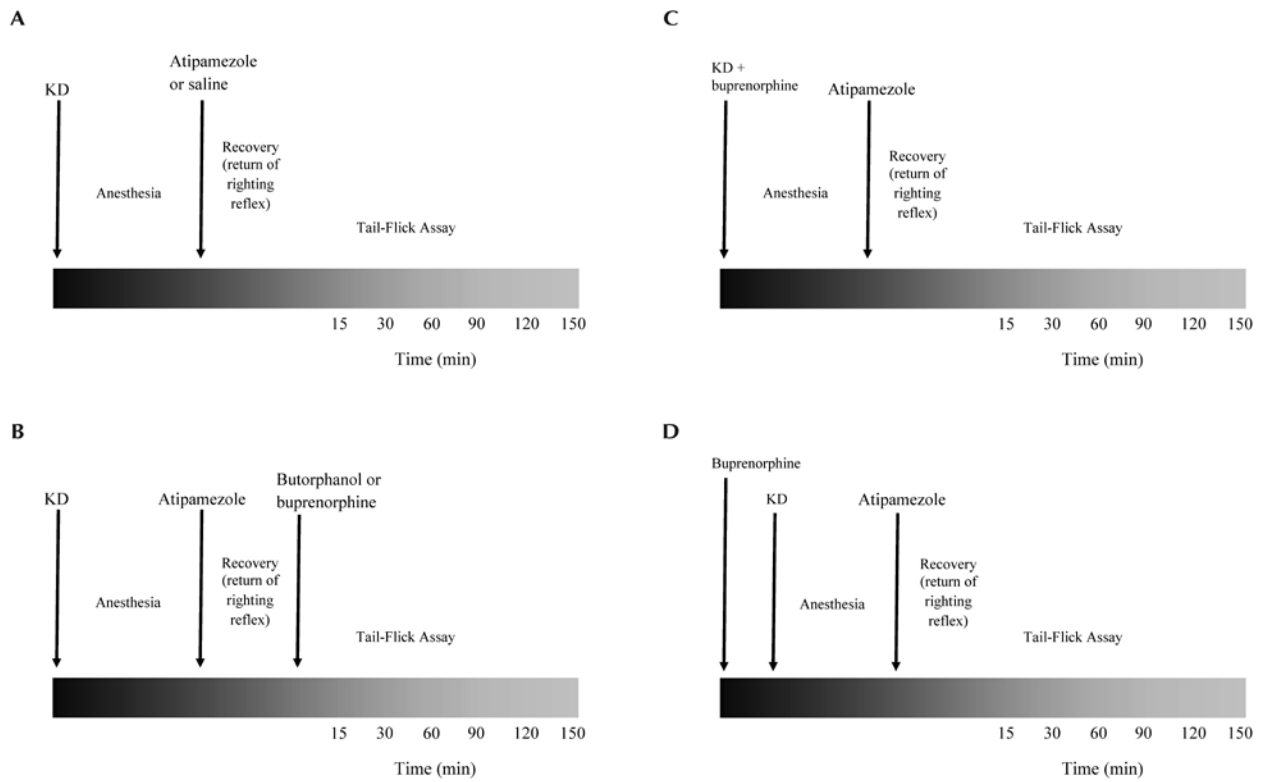

Figure 1. Timeline illustrating procedures performed in KD-anesthetized mice. (A) Atipamezole or saline was administered after 30 min of anesthesia. Animals receiving saline recovered spontaneously from anesthesia. TFL were assessed beginning 15 min after return of the righting reflex. (B) Anesthesia was reversed after 30 min and butorphanol or buprenorphine was administered after return of the righting reflex and TFL measured beginning 15 min after administration of the opioid. (C) Low-dose (0.05 mg/kg) buprenorphine was administered with KD and anesthesia reversed after 30 min. TFL was measured beginning 15 min after return of the righting reflex. (D) Low-dose buprenorphine was injected intraperitoneally 30 min prior to administration of KD. Anesthesia was reversed after 30 min and TFL assessed beginning 15 min after return of the righting reflex.

min after recovery ($n = 30$). The control group of mice recovered spontaneously from KD anesthesia, and TFLs subsequently were measured at 15, 30, 60, 90 and 120 min following recovery ($n = 30$). The time points recorded were: T0, time when KD was administered; T1, time to loss of the righting reflex (that is, when the mouse was unable to right itself after being tipped over gently); T2, time when atipamezole or saline was administered; and T3, time when the righting reflex returned (that is, when the mouse was able to right itself from recumbency).

The onset of anesthesia was calculated as the difference between the time of injection and time at the loss of the righting reflex. The duration of anesthesia for mice for which anesthesia was not reversed was calculated as the difference between the loss and return of the righting reflex. A 30-min period of anesthesia, which began at time point T1, was selected to represent an average period of unconsciousness often required for commonly performed rodent surgeries.

Subsequently the antinociceptive properties of butorphanol and buprenorphine after atipamezole reversal of KD anesthesia were assessed (Figure 1). Mice were divided into 2 groups: KD reversed with atipamezole and followed by butorphanol ($n = 15$) and KD reversed with atipamezole and followed by high-dose buprenorphine ($n = 15$). Atipamezole was administered after a 30-min period of anesthesia. As soon as the mice regained the righting reflex after anesthetic reversal, butorphanol or buprenorphine was administered to the corresponding group. TFL was measured at 15, 30, 60, 90, 120, and 150 min after analgesic drug treatment.

The third aim of the study was to determine the optimal time at which to administer buprenorphine to achieve immediate postoperative analgesia. Naïve mice were allocated into 3 groups: KD followed by atipamezole reversal ($n = 15$); KD with simultaneous buprenorphine (0.1 mg/kg) administration followed by atipamezole reversal ($n = 15$); and buprenorphine (0.1 mg/kg) 30 min prior to anesthetic induction with KD and followed by atipamezole reversal ($n = 15$). Mice were anesthetized and provided with thermal support as described previously. The time at which the pedal withdrawal reflex was lost was recorded, as were time points T0 through T3, as described previously. TFL was measured at 15, 30, 60, 90, 120, and 150 min after the mice regained the righting reflex.

Statistical analysis. Data was analyzed by using Prism statistical software (version 6.02, GraphPad Software, San Diego, CA). TFL was expressed in terms of maximal possible effect (MPE), calculated as follows:[12]

$$\text{MPE}(\%) = \frac{\text{TFL after drug} - \text{baseline TFL} \times 100\%}{10.0\text{ s} - \text{baseline TFL}}$$

All data are expressed as mean ± 1 SD. Data were analyzed by 2-way ANOVA for repeated measures, with time and treatment as the main factors. The Newman–Keuls multiple-comparison test was used to determine which treatments differed at specific time points. Statistical significance was considered to be a P value of less than 0.05.

Results

Effect of atipamezole on reversal of KD anesthesia. KD consistently produced a loss of righting reflex within 1 to 2 min after injection (Table 1). Interestingly, only 60% (36 of 60) of female C57BL/6J mice anesthetized with KD alone lost the pedal withdrawal reflex. Animals that lost the pedal

Table 1. Onset and duration of anesthesia induced with intraperitoneal ketamine–dexmedetomidine (KD)

Group	Onset of anesthesia (min)	Absence of pedal withdrawal reflex/total no. of mice (*n*)	Time to loss of pedal withdrawal reflex (min)	Time to return of righting reflex (min)	Duration of anesthesia (min)
KD plus saline	1.33 ± 0.66	15/30	19.1 ± 7.6	184.4 ± 16.6	184.4 ± 16.6
KD reversed with atipamezole	1.43 ± 0.63	21/30	19.0 ± 6.4	17.4 ± 30.6	46.0 ± 30.5
KD plus buprenorphine and reversed with atipamezole	1.6 ± 0.5	15/15	14.0 ± 3.1	9.7 ± 5.1	38.1 ± 5.2
Buprenorphine plus KD and reversed with atipamezole	1.7 ± 1.4	15/15	9.5 ± 2.9	9.4 ± 3.4	37.8 ± 3.3

Anesthetized mice were injected intraperitoneally with saline or atipamezole 30 min after the onset of anesthesia. Time (min) expressed as mean ± 1 SD.

withdraw reflex needed approximately 19 min from the time of anesthetic injection to lose the reflex. Mice left to recover spontaneously from KD anesthesia, that is, without reversal by atipamezole, remained anesthetized for approximately 3 h. TFL after spontaneous recovery from KD anesthesia revealed moderate antinociception (25% to 50% MPE), which increased approximately 2-fold by 150 min after recovery (Figure 2 A). Anesthetized mice reversed with atipamezole regained the righting reflex 9 to 17 min after the administration of the reversal agent. In addition, TFL at 15 to 60 min after reversal of anesthesia with atipamezole was at or below baseline values (Figure 2 B). TFLs at 15 and 30 min were approximately 0.15 to 0.5 s shorter than baseline latencies. There was a time-dependent increase in TFL from 60 to 150 min after the reversal of anesthesia, but the magnitude of TFL peaked at approximately one-half the magnitude measured in mice that recovered spontaneously from anesthesia.

Effect of atipamezole on TFL. TFL in mice injected with either atipamezole or an equivalent volume of saline did not differ significantly ($P = 0.23$) over time or by treatment (Figure 3). Mean TFLs at 15, 30, and 60 min after treatment with saline or atipamezole were 1.5% to 12.5% shorter than baseline values, that is, as much as 0.5 s faster than the latency measure before drug administration.

Effect of atipamezole on antinociceptive properties of butorphanol and buprenorphine. To ascertain whether atipamezole altered the antinociceptive properties of the opioid analgesics butorphanol and buprenorphine, TFL was measured at specified times after mice were injected with atipamezole followed by butorphanol, low-dose (0.05 mg/kg) buprenorphine, or high-dose (0.1 mg/kg) buprenorphine. Control mice received equivalent volumes of saline followed by butorphanol or buprenorphine. There was no significant difference in TFL value between mice treated with atipamezole and butorphanol compared with saline and butorphanol at 15, 30, and 60 min after treatment ($P = 0.28$, Figure 4). Similarly, TFL values in mice treated with atipamezole or saline followed by low-dose buprenorphine did not differ at 15, 30 and 60 min after analgesic treatment ($P = 0.69$, Figure 5). There was, however, a significant ($P = 0.034$) difference between the TFL at the 15- and 60-min time points after the administration of saline paired with low-dose buprenorphine, such that the TFL at 60 min was 1.6 to 2 times longer than that at the 15-min time point.

Likewise, there were no significant differences in TFL values at 15, 30, 60, 90, and 120 min in mice treated with atipamezole and high-dose (0.1 mg/kg) buprenorphine compared with saline and high-dose (0.1 mg/kg) buprenorphine ($P = 0.68$, Figure 6). Because a time-dependent increase in TFL was noted in the mice treated with low-dose buprenorphine, additional measurements of TFL were obtained at 90 and 120 min after drug administration. There was a significant ($P < 0.0001$) difference between the baseline TFL and those at later time points. Compared with that at the 15-min time point, TFL was significantly higher at 60, 90, and 120 min after the administration of the analgesic. The higher dose of buprenorphine produced greater TFL values than did butorphanol or low-dose buprenorphine at 30 to 120 min after treatment, with the peak TFL of 58% to 64% MPE at 120 min.

Effect of atipamezole on butorphanol and high-dose buprenorphine after KD anesthesia. After the reversal of KD anesthesia, there was a significant ($P < 0.05$) difference between the mice that received atipamezole only and those that received atipamezole followed by butorphanol or high-dose buprenorphine at 30, 60 and 150 min after treatment with analgesic or vehicle (Figure 7). There was a significant ($P < 0.0001$ to $P < 0.05$) time-dependent increase in TFL in the respective treatment groups from 15 to 150 min after reversal of anesthesia. The additional treatment with butorphanol or buprenorphine reversed the negative antinociceptive effect of reversal with atipamezole alone at the 15- and 30-min time points. Furthermore, the TFL in mice treated with 0.1 mg/kg buprenorphine after reversal of KD anesthesia with atipamezole was significantly higher than that of butorphanol-treated animals ($P < 0.05$) and of mice treated with atipamezole alone ($P < 0.001$) at 150 min after reversal.

Effect of preemptive buprenorphine treatment on postanesthetic analgesia. Buprenophine (0.1 mg/kg) administered 30 min prior or at the time of induction of KD anesthesia supported the production of a reliable surgical plane of anesthesia (Table 1). Loss of the pedal withdrawal reflex occurred in 100% of anesthetized mice that received buprenorphine prior to or at the time of anesthesia induction. Furthermore, administering buprenorphine 30 min prior to anesthesia induction reduced the time to absence of the pedal withdraw reflex by approximately 32%. Buprenorphine administered prior to or at the time of anesthesia induction also influenced antinociception after the reversal of anesthesia with atipamezole (Figure 8). There was no significant difference in TFL values in mice that received buprenorphine at the time of anesthesia induction compared with animals that received buprenorphine 30 min prior to the induction, except at the 120-min time point after reversal of anesthesia ($P < 0.05$). TFL was significantly longer at 60 ($P < 0.05$), 90 ($P < 0.001$ to $P < 0.01$), 120 ($P < 0.0001$ to $P < 0.001$) and 150 ($P < 0.0001$) min in mice that received buprenorphine at or prior to induction of anesthesia compared with animals in which KD anesthesia was reversed with atipamezole. When buprenorphine was administered 30 min prior to anesthesia, increased TFL values were detected starting at the 15-min time point after reversal of anesthesia, with the longest TFL achieved at the 150-min time point. Furthermore, administering buprenorphine 30 min prior

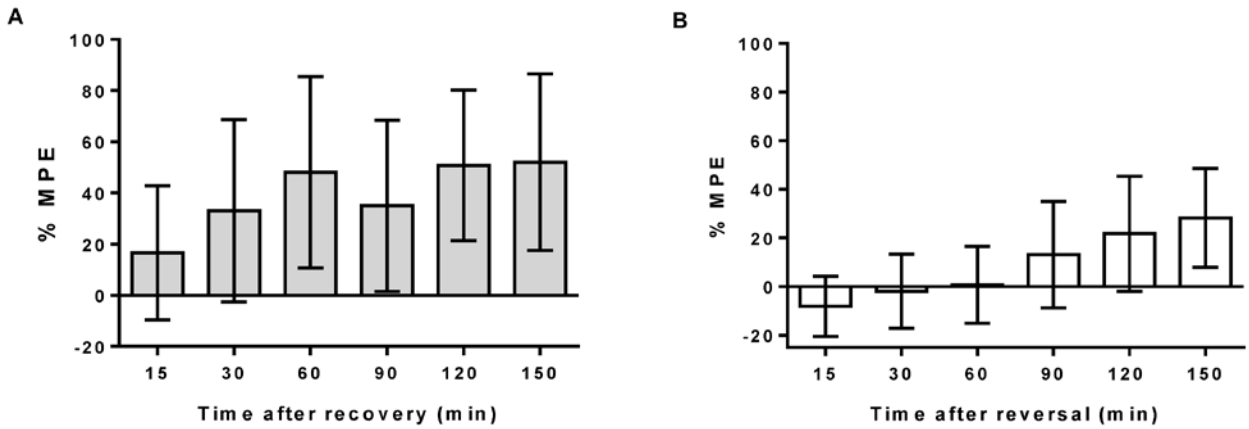

Figure 2. TFL of mice (A) after spontaneous recovery from KD anesthesia or (B) after the reversal of KD anesthesia with atipamezole. Each bar represents the mean ± 1 SD of the group (n = 30). TFL is expressed as the percentage of the maximal possible effect (% MPE). The TFL of the mice prior to administration of the anesthetic was 2.7 ± 0.3 s.

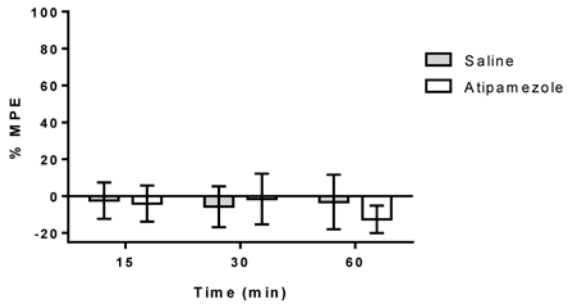

Figure 3. Effect of atipamezole (n = 8) on TFL in mice. Mice in the control group received a similar volume of saline (n = 9). Each bar represents the mean ± 1 SD of the group. TFL is expressed as the percentage of maximal possible effect (% MPE). Time points shown are given as minutes after injection of saline or atipamezole. The TFL of mice prior to drug administration was 2.6 ± 0.2 s.

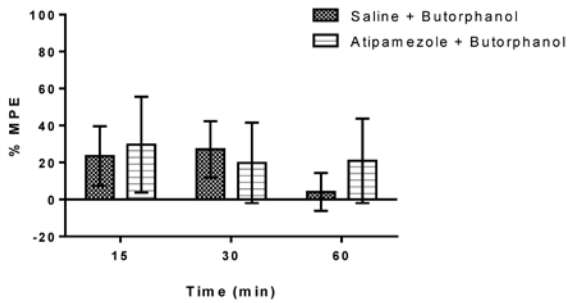

Figure 4. Effect of atipamezole (n = 9) on the antinociceptive properties of butorphanol. The control group received saline (n = 8) and butorphanol. Each bar represents the group mean ± SD. TFL is expressed as the percentage of the maximal possible effect (% MPE). Time points shown are given as minutes after injection of saline or atipamezole. The TFL of mice prior to drug administration was 2.5 ± 0.3 s.

to anesthesia induction produced TFL values that were 4% to 21% higher than those when buprenorphine was administered concurrently with anesthesia and 10% to 53% higher than those when no opioid analgesics were given at all.

Discussion

Atipamezole effectively reversed the anesthetic effects of KD anesthesia, in that female C57BL/6J mice were awake and ambulating, on average, by 17.4 min after the administration of the reversal drug. However, in contrast to that in mice permitted to recover spontaneously from KD anesthesia, postanesthetic antinociception was nonexistent for as long as 90 min after

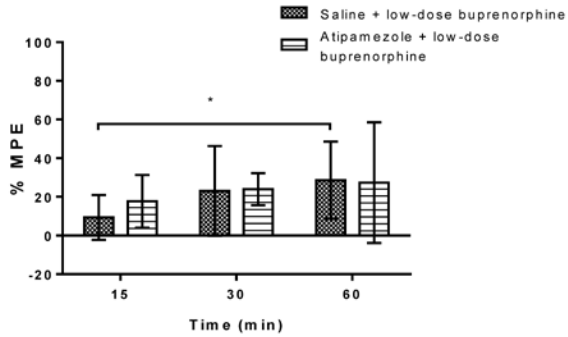

Figure 5. Effects of atipamezole on the antinociceptive properties of low-dose (0.05 mg/kg) buprenorphine. Bars indicate the mean ± 1 SD for each of the groups: atipamezole and low-dose buprenorphine (n = 8) and saline and low-dose buprenorphine (n = 11). TFL is expressed as the percentage of the maximal possible effect (% MPE). Time points shown are given as minutes after injection of saline or atipamezole. The TFL of mice prior to drug administration was 2.4 ± 0.2 s. There was a significant (*, $P < 0.05$) difference between the 15- and 60-min time points after the administration of saline paired with low-dose buprenorphine.

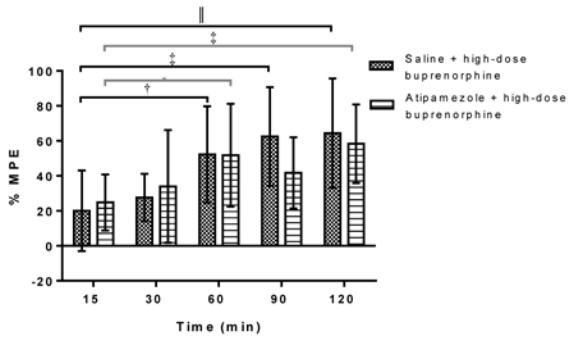

Figure 6. Effects of atipamezole on the antinociceptive properties of high-dose (0.1 mg/kg) buprenorphine. Bars indicate the mean ±1 SD for each of the groups: atipamezole and high-dose buprenorphine (n = 10) and saline and high-dose buprenorphine (n = 9). TFL is expressed as the percentage of the maximal possible effect (% MPE). Time points shown are given as minutes after injection of saline or atipamezole. The TFL of mice prior to drug administration was 2.6 ± 0.3 s. Value is significantly (*, $P < 0.05$; †, $P < 0.01$; ‡, $P < 0.001$; ‖, $P < 0.0001$) different from that at 15 min.

reversal and approximately one-half the magnitude of spontaneously recovering mice thereafter. Moreover, there was a pronociceptive trend, that is, TFL was lower than baseline values at 15 and 30 min after reversal. These findings were not

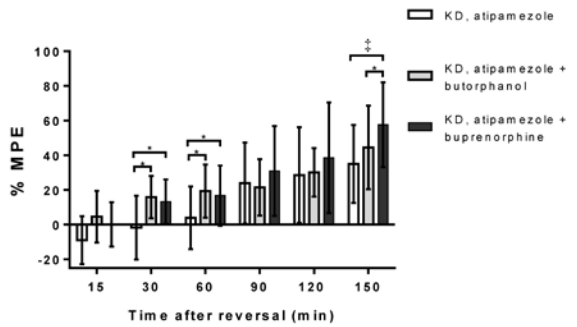

Figure 7. Antinociceptive properties of butorphanol and high-dose (0.1 mg/kg) buprenorphine after reversal of KD anesthesia with atipamezole. Bars indicate the mean ± 1 SD for each of the groups: KD followed by atipamezole (n = 15); KD followed by atipamezole and butorphanol (n = 15); and KD followed by atipamezole and buprenorphine (n = 15). TFL is expressed as percentage as the maximal possible effect (% MPE). Time points shown are given as minutes after reversal of anesthesia. The TFL of mice prior to anesthesia was 2.6 ± 0.3 s. Value is significantly (*, $P < 0.05$; ‡, $P < 0.001$) different between treatment groups.

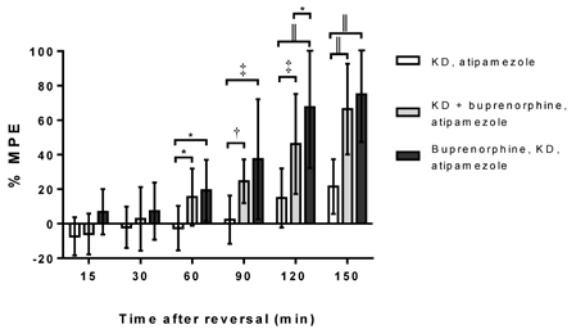

Figure 8. Antinociceptive effects of buprenorphine administered at the time of induction or 30 min prior to the induction of KD anesthesia. Bars indicate the mean ± 1 SD for each of the groups: KD with atipamezole reversal (n = 15); KD with simultaneous buprenorphine administration followed by atipamezole reversal (n = 12); and buprenorphine 30 min prior to anesthetic induction with KD followed by atipamezole reversal (n = 15). TFL is expressed as the percentage of the maximal possible effect (% MPE). The TFL of mice prior to drug administration was 2.5 ± 0.3 s. Value is significantly (*, $P < 0.05$; †, $P < 0.01$; ‡, $P < 0.001$; ‖, $P < 0.0001$) different between treatment groups.

unexpected, because atipamezole is a potent α_2-adrenoceptor antagonist; therefore the analgesic properties of dexmedetomidine were lost after reversal of anesthesia. It was surprising that the ketamine fraction of the anesthetic combination afforded no antinociceptive effect, given that subanesthetic doses of ketamine have analgesic properties in humans and other species.[27] However, N-methyl-D-aspartate receptor antagonists, such as ketamine, are ineffective against acute phasic pain, which is the pain modality assessed by the tail-flick test, and are instead more effective in reducing tonic and chronic types of pain.[34,35] This property of ketamine may explain why an increase in TFL did not occur until 90 to 150 min after KD reversal with atipamezole in the present study.

By comparison, in female C57BL/6J mice permitted to recover spontaneously from KD anesthesia, mild to moderate (20% to 50% MPE) levels of thermal antinociception persisted for as long as 150 min after recovery, indicating that some level of analgesia is present even after recovery from the sedative and anesthetic effects of ketamine and dexmedetomidine. Clearly the potential lack of immediate analgesia suggested by the diminished TFL for as long as 90 min after reversal of anesthesia is of concern from a pain management and animal welfare perspective. The administration—either preemptively or at the time of reversal of anesthesia— of other analgesic drugs not affected by atipamezole likely will prove beneficial in addressing these concerns.

The tail-flick assay was used to evaluate the interaction of the α_2 adrenergic antagonist atipamezole on the antinociceptive properties of butorphanol and buprenorphine after treatment with atipamezole alone or after reversal of KD anesthesia. Evaluation of the antinociceptive effects of atipamezole indicated no statistically significant change in TFL in comparison to that of the control group. However, a trend in the data suggests a pronociceptive effect until 60 min after the administration of the α_2 adrenoceptor antagonist. This finding is similar to that in a study using male Han–Wistar rats, which demonstrated a decrease in TFL below the baseline value after the administration of 1.5 mg/kg atipamezole.[29] We observed a similar reduction in TFL relative to baseline in saline-treated mice in the present study. Although there were no significant differences between treatments or time points, these trends may be explained by habituation of the mice to the tail-flick assay.[2,12] Alternatively an increase in tail temperature over time could explain these findings, because there is an inverse relationship between the temperature of the tail skin and TFL.[2,3] However, this explanation is unlikely given that the ambient temperature in the procedure room was maintained at 21 °C throughout the study and the mice were not provided supplemental heat during this portion of the study. Overall, atipamezole had little effect on the thermal antinociceptive properties of butorphanol or buprenorphine when the analgesic drugs were evaluated alone or in combination with KD anesthesia.

In our study, butorphanol had less of an antinociceptive effect than did buprenorphine. This finding is in agreement with previous studies, which evaluated antinociception by using the tail-flick and hotplate tests in ICR mice and found lower levels of antinociception after butorphanol and intermediate levels after buprenorphine relative to those due to morphine.[15] Interestingly, we noted both time- and dose-dependent increases in TFL after treatment with buprenorphine. In mice treated with low-dose (0.05 mg/kg) buprenorphine, TFL progressed from a mild (<25% MPE) to moderate (<60% MPE) response over 60 min after treatment. In contrast, the higher dose of buprenorphine (0.1 mg/kg) resulted in a gradual increase in thermal antinociception from moderate (25% to 50% MPE) to marked (>50% MPE) over the same time period. Similar time- and dose-dependent antinociception with the peak effect at 60 min occurred in Sprague–Dawley rats given buprenorphine by the subcutaneous route and tested by using an aversive electrical stimulus.[11] Investigations of the thermal antinociceptive properties of buprenorphine in adult female Clun crossbred ewes similarly demonstrated a time- and dose-dependent antinociceptive effect of the drug.[32,44] In this study, buprenorphine administered intravenously at a dose rate of 1.5 µg/kg produced a gradual increase in the sheep's thermal threshold, reaching maximal effect at 40 min after drug administration.[44] Buprenorphine administered at 6 µg/kg IV to the sheep produced greater levels of antinociception over a shorter time course than did the lower dose.[32,44] These findings parallel those of the current study, in which higher magnitudes of thermal antinociception were produced with high-dose (0.1 mg/kg) compared with low-dose (0.5 mg/kg) buprenorphine in female C57BL/6J mice. Furthermore, no antinociceptive activity in the thermal test could be detected in sheep during the first 60 min after injection, coinciding with the period of time in which drug plasma levels were high.[32] Although dramatically different species, the 60-min delay in reaching peak levels of thermal antinociception in the mice of the current study and sheep of

previous studies may be explained by the pharmacodynamics and opioid receptor kinetics of buprenorphine. The slow onset and long duration of buprenorphine's analgesic activity have been suggested to be due to its unique receptor association–dissociation kinetics, in which the binding to and dissociation from the μ opioid receptor is slow.[5,8,46] Furthermore, buprenorphine has opposite effects (agonist or antagonist) at μ and κ receptors, depending on the dose administered.[37,39]

Administration of butorphanol or buprenorphine at the time of anesthetic reversal shortened the duration of no measureable thermal antinociceptive effect to 30 min after reversal and produced greater magnitudes of antinociception at later time points compared with those of mice that did not receive an opioid after anesthetic reversal. However, the delay in antinociceptive response between administration of the analgesic and a detectable increase in TFL was unexpected and troubling. Reversal of anesthesia with atipamezole in animals undergoing a surgical procedure may lead to a period during which there is no analgesia or, even worse, enhanced pain sensitivity despite administration of potent analgesics at the conclusion of the procedure. Perhaps the interaction of multiple drugs (that is, ketamine, dexmedetomidine, atipamezole, and butorphanol or buprenorphine) during this time resulted in the lag in the antinociceptive effect of the 2 opioid drugs. We demonstrated that the administration of atipamezole with butorphanol or buprenorphine in unanesthetized mice did not alter the antinociceptive effects of either opioid. In one report, ketamine had an additive effect on TFL when combined with fentanyl or morphine.[10] In another investigation in Sprague–Dawley rats, subanesthetic doses (1.5 to 3 mg/kg) of ketamine reversed the pronociceptive effects of ultra-low-dose (20 μg/kg) buprenorphine and prolonged the time to development of tolerance to the analgesic effects of buprenorphine.[43] It would be interesting to investigate atipamezole reversal of dexmedetomidine only combined with opioid analgesics. Another possible explanation for the delay in antinociceptive effect of butorphanol or buprenorphine may be the altered absorption and distribution of the drugs in mice anesthetized previously.

In subsequent experiments, high-dose (0.1 mg/kg) buprenorphine was administered 30 min prior to or at the time of induction of anesthesia in female C57BL/6J mice, and thermal antinociception was present earlier and reached higher levels after reversal of anesthesia with atipamezole. Administering buprenorphine preemptively (that is, 30 min prior to induction) resulted in the greatest magnitude (>75% MPE) of antinociception after anesthetic reversal. Administering the analgesic prior to anesthesia may have permitted unaltered absorption and distribution of the drug, resulting in higher plasma levels of the drug during the postreversal period. An added benefit of administering buprenorphine prior to or at the time of anesthesia induction was a surgical plane of anesthesia, as determined by loss of the pedal withdrawal reflex, uniformly observed in all mice. In contrast, loss of the pedal withdrawal reflex was variable in mice anesthetized with KD alone. This finding is in agreement with a recent study, which found that the pedal withdrawal reflex was less reliably abolished in C57BL/6N mice anesthetized with KD compared with ketamine–medetomidine.[7] In addition, time to loss of tail pinch and forelimb and hindlimb pedal withdrawal reflexes in female BALB/cJ mice anesthetized with ketamine–medetomidine was highly variable, requiring as long as 40 min after loss of the righting reflex in some animals.[1] Addition of acepromazine to the anesthetic regimen shortened the lag time until all 3 reflexes were lost.[1] Perhaps more mice in our study would have lost the pedal withdrawal reflex over time, but even some of the animals that recovered spontaneously from anesthesia did not lose the reflex.

Moreover, when buprenorphine was administered before or at the time of anesthetic induction, the time to return of the righting reflex after atipamezole was approximately half the duration when buprenorphine was administered at the time of reversal. A likely explanation for this observation is that any sedative effects of buprenorphine, when administered before or at induction, had diminished by the time the atipamezole was administered such that the analgesic did not prolong the anesthesia time.

We chose to investigate the effects of atipamezole on the thermal antinociceptive properties of butorphanol and buprenorphine because they often are administered to rodents postoperatively for their potent analgesic effects. Butorphanol exhibits partial agonist and antagonist activity at the μ opioid receptor and agonist activity at the κ opioid receptor.[13] Buprenorphine is a partial μ agonist that is commonly used for postoperative analgesia due to its long duration of action and minimal adverse side effects.[13] Atipamezole partially altered the thermal antinociceptive effects of butorphanol in adult male Sprague–Dawley rats as measured by tail withdrawal from a 50 °C water bath.[25] Assuming that a relationship between descending noradrenergic and serotonergic pathways exists, the authors postulated that atipamezole inhibits the activity of serotonergic pathways by blocking α_2 adrenoceptors.[24] Consequently, the authors further speculated that because κ opioid analgesia is dependent on central serotinergic pathways, butorphanol antinociception likely was inhibited via the inactivation of serotonergic pathways.[25] It was unclear whether this finding was receptor-specific or species-specific.

If indeed the atipamezole antagonism of butorphanol-induced thermal antinociception is due to inhibition of the opioid's κ receptor-induced analgesic effects, we postulated that atipamezole would have no effect on the antinociceptive properties of buprenorphine, given its μ opioid receptor specificity. We found that atipamezole did not modulate the thermal antinociceptive profile of either butorphanol or buprenorphine. The discrepancy between our study and a previous one[24] may be due to a difference in the distribution and pharmacology of serotonergic receptors in rats compared with mice.[21] Alternatively, the authors of the previous study[25] suggested that a potential pronociceptive effect of atipamezole may have diminished the antinociceptive effects of butorphanol. Furthermore, stimulus intensity also may determine the magnitude of TFL. A dose-dependent decrease in TFL developed in Han–Wistar rats treated with atipamezole when low and intermediate stimulus intensities were used in testing, but there was no effect on TFL when a high intensity stimulus was used.[29] Because the authors of the previously cited study[24] did not measure TFL after the administration of atipamezole alone, we included this evaluation in our study and accordingly identified a time-dependent procociceptive trend in mice treated with atipamezole; however, a similar but less pronounced trend was also seen in mice treated with saline. Furthermore, we did not see differences in butorphanol or buprenorphine antinociception when paired with atipamezole or saline, therefore, a pronociceptive effect of atipamezole cancelling or diminishing the antinociceptive effect of either opioid is unlikely.

The authors of the earlier study[24] also considered that various states of arousal, which were not assessed, could influence TFL; however, based on other work, they concluded that the arousal state did not influence butorphanol TFL.[20,25] In the current study, tail-flick testing in mice previously anesthetized with KD did not

take place until 15 min after return of the righting reflex, therefore state of arousal did not influence TFL. Similar to our results, other colleagues concluded that buprenorphine produced an intermediate analgesic effect in mice according to the tail-flick assay.[15] The results of the present investigation are in agreement with several previous studies that found butorphanol provided weak analgesic effects compared with other opioids in male Hsd:ICR and male ND4 Swiss Webster mice.[15,16] Butorphanol analgesia in male Hsd:ICR mice and male Hsd:SD rats only lasts 1 to 2 h and therefore requires frequent dosing, which is often impractical in a laboratory setting.[15] Compared with butorphanol, buprenorphine has a longer duration of action, lasting 3 to 5 h in Hsd:ICR mice and 6 to 8 h in Hsd:SD rats.[15] Our findings further support those previous studies, given that TFL increased with time after buprenorphine treatment alone or when administered before, concurrently, or after KD anesthesia in female C57BL/6J mice.

Consistent with our reported results, we made several observations of clinical relevance during the present study. Intraperitoneal administration of butorphanol alone produced marked sedation in female C57BL/6J mice, as evidenced by their reduced activity and decreased movement within the cage. In contrast, there were no clinical signs of sedation after buprenorphine administration. The combination of ketamine with dexmedetomidine rapidly induced anesthesia, with loss of the righting reflex within 2 min of injection. Administration of buprenorphine before or at the same time as KD did not alter the onset of anesthesia. After a 30-min period of anesthesia, atipamezole effectively reversed the anesthetic effects of KD, with mice regaining their righting reflex approximately 10 min after administration of the antagonist. After reversal, mice were awake and ambulatory but remained mildly sedate as they moved about the cage. Mice that were not given the antagonist drug recovered spontaneously approximately 3 h after injection with KD. These findings agree with previous reports, which found that the administration of atipamezole after ketamine–medetomidine anesthesia produced a more rapid recovery.[1,9] Interestingly, female C57BL/6J mice treated with buprenorphine either prior to or with KD regained their righting reflex faster after atipamezole reversal than they did when KD was reversed with atipamezole but without opioids.

Another observation of clinical importance was that only 63% of female C57BL/6J mice anesthetized with KD lost their pedal withdrawal reflex over a 30-min period of anesthesia. In the mice that lost the pedal withdrawal reflex, it took approximately 19 min after KD administration to develop absence of the reflex. This observation is consistent with previous investigations, which showed inconsistent loss of the pedal withdrawal reflex in male and female C57BL/6N, and female inbred BALB/cJ mice, respectively, when anesthetized with a combination of medetomidine or dexmedetomidine with ketamine.[1,7] In contrast, 100% of the mice treated with buprenorphine 30 min prior to or at the time of induction of KD anesthesia lost their pedal withdrawal reflex. Therefore, the anesthetic combination of KD plus buprenorphine reliably produced a surgical plane of anesthesia in female C57BL/6J mice. Furthermore, when buprenorphine was administered 30 min prior to anesthesia, a surgical plane of anesthesia was reached in half the time (approximately 9.5 min) that was required by mice anesthetized with KD and buprenorphine or KD alone. This finding is in agreement with an earlier report in which propofol-anesthetized rats given buprenorphine prior to anesthesia required a smaller total dose of propofol to maintain a surgical plane of anesthesia and had improved recovery scores compared with those in rats that did not receive buprenorphine.[33]

The present study had several limitations. We used the tail-flick assay to investigate the interaction of an α_2 adrenoceptor agonist and antagonist in the presence of ketamine anesthesia and their interaction with mixed compared with pure opioid analgesics in female C57BL/6J mice. Although fewer than half of the mice failed to reach a surgical plane of anesthesia, they were not excluded from the tail-flick assay after anesthetic recovery, because the primary objective of the study was to evaluate antinociception after reversal of anesthesia. It is important to note that the tail-flick assay is a pure measure of thermal nociception, whereas the pedal withdrawal reflex, a commonly used indicator of surgical anesthesia, simultaneously stimulates mechanoreceptors as well as mechanonociceptors. Although analgesiometric tests provide a way of measuring the resultant antinociceptive effects of drug interactions, the neurologic mechanisms involved in the assay may not be equivalent to those responsible for clinical pain.[39] Therefore, our study results should be extrapolated with caution to animals experiencing postoperative pain, given that the tail-flick test uses a controlled, limited, pure noxious stimulus in contrast to the complex, multifaceted unlimited stimulus resulting from surgery. In addition, the results of this study are not necessarily translatable to all strains and sexes of mice. Furthermore, multiple studies have reported significant variations in response to analgesics depending on strain and sex.[30,36,39,40] We chose to use C57BL/6J mice in our study, because they are a commonly used inbred strain in pain-related studies and are widely used as a genetic background for many lines of genetically engineered mice.[45] Finally, a prestudy power analysis indicated that a much larger sample size (approximately 300 mice per experimental group) may be needed to detect significant differences. Accommodating this sample size was impractical, and experimental groups of this size are not used with the tail-flick assay.[2] Instead, we used a sample size of at least 8 mice per group and dose, as recommended in the standard protocol for tail-flick assay.[2] It is possible that statistically significant differences, as compared with trends in the data, could have been detected by using a larger sample size. Moreover, the researcher who performed the analgesic testing was not masked to the treatment group in which the animals were assigned—another limitation of the current study.

In conclusion, atipamezole effectively and reliably reversed the anesthetic effects of KD anesthesia in female C57BL/6J mice. Predictably, atipamezole antagonized the antinociceptive, and by extension, analgesic effects of dexmedetomidine. However, atipamezole does not interfere with the antinociceptive effects of butorphanol or buprenorphine, even after reversal of anesthesia in these mice. In light of the results of this study, for maximum postanesthetic antinociception, we recommend the administration of buprenorphine either 30 min prior to or at the time of KD anesthesia induction to provide a defined period of surgical anesthesia that is easily reversed by atipamezole.

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References

1. **Baker NJ, Schofield JC, Caswell MD, McLellan AD.** 2011. Effects of early atipamezole reversal of medetomidine–ketamine anesthesia in mice. J Am Assoc Lab Anim Sci **50:**916–920.
2. **Bannon AW, Malmberg AB.** 2007. Models of nociception: hot-plate, tail-flick, and formalin tests in rodents. Curr Protoc Neurosci **Chapter 8**:Unit 8.9.

3. **Berge OG, Garcia-Cabrera I, Hole K.** 1988. Response latencies in the tail-flick test depend on tail skin temperature. Neurosci Lett **86:**284–288.
4. **Blaha MD, Leon LR.** 2008. Effects of indomethacin and buprenorphine analgesia on the postoperative recovery of mice. J Am Assoc Lab Anim Sci **47:**8–19.
5. **Boas RA, Villiger JW.** 1985. Clinical actions of fentanyl and buprenorphine. The significance of receptor binding. Br J Anaesth **57:**192–196.
6. **Browning S, Lawrence D, Livingston A, Morris B.** 1982. Interactions of drugs active at opiate receptors and drugs active at α2 receptors on various test systems. Br J Pharmacol **77:**487–491.
7. **Burnside WM, Flecknell PA, Cameron AI, Thomas AA.** 2013. A comparison of medetomidine and its active enantiomer dexmedetomidine when administered with ketamine in mice. BMC Vet Res **9:**48.
8. **Cowan A, Doxey JC, Harry EJ.** 1977. The animal pharmacology of buprenorphine, an oripavine analgesic agent. Br J Pharmacol **60:**547–554.
9. **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. Lab Anim **32:**18–22.
10. **Dambisya YM, Lee TL.** 1994. Antinociceptive effects of ketamine–opioid combinations in the mouse tail-flick test. Methods Find Exp Clin Pharmacol **16:**179–184.
11. **Dum JE, Herz A.** 1981. In vivo receptor binding of the opiate partial agonist buprenorphine correlated with its agonistic and antagonistic actions. Br J Pharmacol **74:**627–633.
12. **Ehrenpreis S, Neidle A.** 1975. Methods in narcotics research. New York (NY): M Dekker.
13. **Fish RE, Brown MJ, Danneman PJ, Karas AZ**. 2008. Anesthesia and analgesia in laboratory animals, 2nd ed. American College of Laboratory Animal Medicine series. Boston (MA): Elsevier Academic Press.
14. **Flecknell PA.** 1996. Laboratory animal anaesthesia: a practical introduction for research workers and technicians. San Diego (CA): Academic Press.
15. **Gades NM, Danneman PJ, Wixson SK, Tolley EA.** 2000. The magnitude and duration of the analgesic effect of morphine, butorphanol, and buprenorphine in rats and mice. Contemp Top Lab Anim Sci **39:**8–13.
16. **Garner HR, Burke TF, Lawhorn CD, Stoner JM, Wessinger WD.** 1997. Butorphanol-mediated antinociception in mice: partial agonist effects and μ receptor involvement. J Pharmacol Exp Ther **282:**1253–1261.
17. **Grant GJ, Zakowski MI, Vermeulen K, Langerman L, Ramanathan S, Turndorf H.** 1993. Assessing local anesthetic effect using the mouse tail-flick test. J Pharmacol Toxicol Methods **29:**223–226.
18. **Green CJ.** 1979. Animal anaesthesia. London (UK): Laboratory Animals.
19. **Hahn N, Eisen RJ, Eisen L, Lane RS.** 2005. Ketamine–medetomidine anesthesia with atipamezole reversal: practical anesthesia for rodents under field conditions. Lab Anim (NY) **34:**48–51.
20. **Hedenqvist P, Roughan JV, Flecknell PA.** 2000. Sufentanil and medetomidine anaesthesia in the rat and its reversal with atipamezole and butorphanol. Lab Anim **34:**244–251.
21. **Hirst WD, Abrahamsen B, Blaney FE, Calver AR, Aloj L, Price GW, Medhurst AD.** 2003. Differences in the central nervous system distribution and pharmacology of the mouse 5-hydroxytryptamine-6 receptor compared with rat and human receptors investigated by radioligand binding, site-directed mutagenesis, and molecular modeling. Mol Pharmacol **64:**1295–1308.
22. **Iglesias V, Alguacil LF, Alamo C, Cuenca E.** 1992. Effects of yohimbine on morphine analgesia and physical dependence in the rat. Eur J Pharmacol **211:**35–38.
23. **Institute for Laboratory Animal Research.** 2011. Guide for the care and use of laboratory animals, 8th ed. Washington (DC): National Academies Press.
24. **Jang HS, Choi HS, Lee SH, Jang KH, Lee MG.** 2009. Evaluation of the anaesthetic effects of medetomidine and ketamine in rats and their reversal with atipamezole. Vet Anaesth Analg **36:**319–327.
25. **Jang HS, Lee MG.** 2009. Atipamezole changes the antinociceptive effects of butorphanol after medetomidine–ketamine anaesthesia in rats. Vet Anaesth Analg **36:**591–596.
26. **Jeong Y, Holden JE.** 2008. Commonly used preclinical models of pain. West J Nurs Res **30:**350–364.
27. **Joel S, Joselyn A, Cherian VT, Nandhakumar A, Raju N, Kaliaperumal I.** 2014. Low-dose ketamine infusion for labor analgesia: a double-blind, randomized, placebo controlled clinical trial. Saudi J Anaesth **8:**6–10.
28. **Jouber KE, Serfontein T, Scantlebury M, Manjerovice MB, Bateman PW, Bennett NC, Waterman JM.** 2011. Determination of an optimal dose of medetomidine–ketamine–buprenorphine for anaesthesia in the Cape ground squirrel (*Xerus inauris*). J S Afr Vet Assoc **82:**94–96.
29. **Kauppila T, Jyväsjärvi E, Hämäläinen MM, Pertovaara A.** 1998. The effect of a selective α2 adrenoceptor antagonist on pain behavior of the rat varies depending on experimental parameters. Pharmacol Biochem Behav **59:**477–485.
30. **Kest B, Wilson SG, Mogil JS.** 1999. Sex differences in supraspinal morphine analgesia are dependent on genotype. J Pharmacol Exp Ther **289:**1370–1375.
31. **Kim MS, Jeong SM, Park JH, Nam TC, Seo KM.** 2004. Reversal of medetomidine–ketamine combination anesthesia in rabbits by atipamezole. Exp Anim **53:**423–428.
32. **Nolan A, Livingston A, Waterman AE.** 1987. Investigation of the antinociceptive activity of buprenorphine in sheep. Br J Pharmacol **92:**527–533.
33. **Penderis J, Franklin RJ.** 2005. Effects of pre- versus postanaesthetic buprenorphine on propofol-anaesthetized rats. Vet Anaesth Analg **32:**256–260.
34. **Petrenko AB, Yamakura T, Askalany AR, Kohno T, Sakimura K, Baba H.** 2006. Effects of ketamine on acute somatic nociception in wild-type and N-methyl-D-aspartate (NMDA) receptor ε1 subunit knockout mice. Neuropharmacology **50:**741–747.
35. **Petrenko AB, Yamakura T, Sakimura K, Baba H.** 2014. Defining the role of NMDA receptors in anesthesia: are we there yet? Eur J Pharmacol **723:**29–37.
36. **Pick CG, Cheng J, Paul D, Pasternak GW.** 1991. Genetic influences in opioid analgesic sensitivity in mice. Brain Res **566:**295–298.
37. **Pick CG, Peter Y, Schreiber S, Weizman R.** 1997. Pharmacological characterization of buprenorphine, a mixed agonist–antagonist with κ3 analgesia. Brain Res **744:**41–46.
38. **Plummer JL, Cmielewski PL, Gourlay GK, Owen H, Cousins MJ.** 1991. Assessment of antinociceptive drug effects in the presence of impaired motor performance. J Pharmacol Methods **26:**79–87.
39. **Roughan JV, Flecknell PA.** 2002. Buprenorphine: a reappraisal of its antinociceptive effects and therapeutic use in alleviating postoperative pain in animals. Lab Anim **36:**322–343.
40. **Semenova S, Kuzmin A, Zvartau E.** 1995. Strain differences in the analgesic and reinforcing action of morphine in mice. Pharmacol Biochem Behav **50:**17–21.
41. **Sinclair MD.** 2003. A review of the physiological effects of α2 agonists related to the clinical use of medetomidine in small animal practice. Can Vet J **44:**885–897.
42. **Stokes EL, Flecknell PA, Richardson CA.** 2009. Reported analgesic and anaesthetic administration to rodents undergoing experimental surgical procedures. Lab Anim **43:**149–154.
43. **Wala EP, Holtman JR.** 2011. Buprenorphine-induced hyperalgesia in the rat. Eur J Pharmacol **651:**89–95.
44. **Waterman AE, Livingston A, Amin A.** 1991. Further studies on the antinociceptive activity and respiratory effects of buprenorphine in sheep. J Vet Pharmacol Ther **14:**230–234.
45. **Wen T, Ansonoff MA, Pintar JE.** 2009. The tail pigmentation pattern of C57BL/6J mice affects nociception–pain quantification in the tail flick test. Eur J Pain **13:**564–567.
46. **Yu S, Zhang X, Sun Y, Peng Y, Johnson J, Mandrell T, Shukla AJ, Laizure SC.** 2006. Pharmacokinetics of buprenorphine after intravenous administration in the mouse. J Am Assoc Lab Anim Sci **45:**12–16.