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Rapid Recovery and Short Duration Anesthesia after Low Dose Ketamine and High Dose Dexmedetomidine in Rhesus Macaques (*Macaca mulatta*)

Kristin E Killoran,[1] Courtney A Walsh,[2] Jennifer L Asher,[1] Molly B Tarleton,[1] and Steven R Wilson[1,*]

Anesthesia in rhesus macaques is required for many procedures. Although ketamine is the backbone of most anesthetic protocols, tolerance to the drug can develop, resulting in the need for higher doses to provide sufficient restraint. Combination with other drugs, such as α-agonists, can be ketamine-sparing, providing for sufficient restraint at lower ketamine doses. In addition, because α-agonists are reversible, recovery from anesthesia has the potential to be much shorter. We hypothesized that use of a low dose of ketamine with a high dose of dexmedetomidine, an α2 receptor selective agonist, in male and female rhesus macaques less than 15 y of age would provide adequate anesthesia for short procedures and that recovery would be faster than in macaques given a higher dose of ketamine (10 mg/kg) alone. We found that the combination, in conjunction with atipamezole for reversal, provided smooth induction of anesthesia and significantly shorter recovery time than did ketamine alone, with no significant effects of sex. The combination of low dose ketamine and high dose dexmedetomidine also provided a 30-min window of anesthesia with analgesia sufficient for mild to moderately painful procedures.

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Anesthesia in rhesus macaques is used for physical examinations, transfer into shipping containers, ultrasound examinations, and blood collection, among other procedures. In colonies in which behavioral training for voluntary examinations and blood collection is unavailable, every animal will require anesthesia at least annually and some much more frequently, depending on the nature of the research. Ketamine is the standard drug in many anesthetic protocols in rhesus macaques. However, tolerance to ketamine can develop,[5,13] especially in individuals who undergo many anesthetic events due to advanced age or study collection intensity. Ketamine-induced anesthesia is characterized by muscle tremors and rigidity with preserved airway reflexes.[1,18] Furthermore, recovery from ketamine anesthesia can be unpredictable in both duration and quality.[1]

Two highly desirable features of anesthesia are rapid and smooth transition to both immobilization and recovery. Rapid and smooth recovery is most easily achieved if the anesthetic can be reversed pharmacologically. The use of ketamine in combination with reversible drugs (for example, xylazine and dexmedetomidine) has been reported.[10,18] Dexmedetomidine, an α2 selective agonist, provides sedation with excellent muscle relaxation, but α-agonists alone are insufficient for safe anesthesia of rhesus macaques.[18] We tested whether treatment with a lower dose of ketamine combined with a high dose of dexmedetomidine could result in rapid, smooth induction of anesthesia and recovery in male and female rhesus macaques less than 15 y of age. We compared this combination to a standard 10 mg/kg dose of ketamine alone. We also determined the duration of anesthesia and analgesia provided by the combination as demonstrated by a negative toe pinch response. We hypothesized that a combination of low dose ketamine and high dose dexmedetomidine would provide a more rapid recovery than did ketamine alone. We also hypothesized that the combination would provide anesthesia and analgesia sufficient for mild to moderately painful procedures of short duration.

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[1]Department of Comparative Medicine, Yale University School of Medicine, New Haven, Connecticut; [2]Atlantic Veterinary College, University of Prince Edward Island, Charlottetown, Prince Edward Island, Canada
[*]Corresponding author. Email: steven.r.wilson@yale.edu

Materials and Methods

Animals. Thirty-six rhesus macaques (9 female, 27 male) less than 15 y of age (range of 4.4-12.3, mean of 7.7 mean and median of 7.8) were included in this study. Before enrollment in this study, we reviewed previous anesthetic records for past complications, including the need for a high ketamine dose, the need to use alternative drug combinations to achieve necessary levels of anesthesia, and poor recoveries. Only animals with no such notations in their medical history were included. Animals were housed in Yale Animal Resources Center's AAALAC-accredited facilities. Animals were enrolled in Yale University Institutional Animal Care and Use Committee-approved protocols for neurobiology research, diagnostic radiology, or breeding. Semiannual physical examinations and intradermal testing for *Mycobacterium tuberculosis* and *bovis* were performed in conjunction with annual complete blood count, serum biochemistry profile, and serology for simian immunodeficiency virus, simian retrovirus type D, simian T-cell leukemia virus, measles virus, and *Macacine herpesvirus-1* (Intuitive Biosciences, Madison, WI). All animals were free of clinical disease at time of testing. Animal Biosafety Level 2 practices were employed throughout the study. Macaques were fed one of 2 commercially

available primate diets (Teklad 8714, Envigo, Indianapolis, IN or LabDiet 5045, LabDiet, St Louis, MO) supplemented with fresh fruits and vegetables daily and food enrichment items were provided for psychologic wellbeing, behavioral testing, or both (for example dried fruit, seed and nut mixes, cereals, peanut butter, peanuts, PrimaTreats [BioServ, Frenchtown, NJ], honey, etc.). The latter did not comprise a substantial portion of the daily caloric intake. All animals were housed in stainless steel caging (Allentown, Allentown, NJ) in pairs or trios whenever possible. Various toys such as stainless-steel mirrors and plastic or rubber manipulanda were provided continuously on a rotating schedule for psychologic wellbeing enhancement. Rooms were on a 12:12 light cycle, temperature was maintained at 72 ± 2 °F (22.2 ± 1.1 °C), and relative humidity at 50 ± 10%.

Anesthetic agents. Doses used were empirically determined within our veterinary group. Ketamine (Ketaject 100 mg/mL, Henry Schein, Portland, ME) was administered intramuscularly (IM) at a dose of either 10 mg/kg bodyweight alone or 1.5 mg/kg body weight when combined with dexmedetomidine. Dexmedetomidine (Dexdomitor, 0.5 mg/mL, Zoetis, Parsippany, NJ) was administered IM at a dose of 0.03 mg/kg in combination with ketamine. Atipamezole (Antisedan, 5.0 mg/mL, Zoetis, Parsippany, NJ) was administered IM at a dose of 0.3 mg/kg to reverse the effects of dexmedetomidine. Drugs were administered using squeeze-back cages.

Induction, duration, and recovery from anesthesia. Time to sedation and time to recovery were recorded for all animals. Time to sedation was defined as the elapsed time from injection of drug(s) to the time the animal could be safely removed from the cage. Time to recovery was defined as the elapsed time from return to cage (ketamine alone) or return to cage with concomitant atipamezole administration IM (ketamine plus dexmedetomidine) to the time at which the animal was sufficiently recovered to allow full access to one quarter of a condominium caging unit (4.3 square feet). Criteria of recovery included perching with no visible instability, no remaining nystagmus, and no remaining ataxia. In animals for whom time to sedation and recovery were being assessed, the duration of removal from home cage to return to home cage ranged from approximately 10 to 15 min. While anesthetized, animals were weighed, had tuberculin injected intradermally in one eyelid, and underwent a physical exam. Some individuals were treated with ivermectin (Noromectin, Norbrook Laboratories, Newry, Northern Ireland), had rectal cultures taken, or had blood drawn for CBC or serum chemistry. During anesthesia, 100% O_2 was provided by facemask. Due to the brief duration of anesthesia, only temperature, heart rate, and respiratory rate were recorded. In animals for whom depth and duration of ketamine plus dexmedetomidine anesthesia were being assessed, time from IM injection to sedation was recorded. Animals were then placed on a recirculating warm water blanket at 98 to 104 °F (36.7 to 40 °C) and covered with a Bair Hugger (3M, St Paul, MN) blanket at 100.4 to 109.4 °F (38 to 43 °C). A pulse oximeter probe was placed on a finger while 100% O_2 was provided by facemask. Heart rate, oxygen saturation, and response to a hemostat closed to one click on the webbing between the hallux and adjacent digit of a hind foot were recorded every 5 min for 30 min. After 30 or 45 min, animals were returned to their cages and given atipamezole IM.

Statistical analysis. A priori sample size estimates from pilot data using G*Power[4] determined that 18 individuals per group were necessary to detect significant differences in the time to sedation and recovery for the ketamine and ketamine plus dexmedetomidine groups (power, 0.95; groups, 2; a, 0.05). Sex was disproportionately distributed across the 2 drug experimental groups in the data sample (N_{female}= 9, N_{male}= 27). As a result, the number of male cases were matched to the number of female cases by randomly selecting a proportionate number of male ketamine (n = 4) and male ketamine plus dexmedetomidine (n = 5) groups. Data were analyzed using SPSS software (SPSS 26, IBM, Armonk, NY). Mann–Whitney U tests were conducted to detect differences between time to sedation and time to recovery for the ketamine and ketamine plus dexmedetomidine groups and to test for a difference in sedation and recovery times by sex. In instances in which the distributions of the dependent variables (time to sedation or time to recovery) were not similar across our groups, mean-ranks were used to conduct the analyses. Measures of effect size were calculated by hand. In all analyses, a P value of 0.05 was used to determine significance.

Results

Time to sedation. Male and female rhesus macaques less than 15 y of age (range, 4.4-12.3; mean, 7.7; median, 7.8) were anesthetized with either ketamine alone or ketamine plus dexmedetomidine. We compared the time to sedation between the 2 groups. The median time to sedation in ketamine-treated animals was 6.0 ±1.4 minutes. The median time to sedation in ketamine plus dexmedetomidine-treated animals was 5.5 ± 1.6 min. These times were not significantly different (U = 124.5, z = -1.200, r = 0.20, p = 0.239) (Figure 1 A). Males and females showed no significant difference in the median time to sedation (U = 32, z = -0.758, r = 0.18, p = 0.489) (Figure 1 B).

Time to recovery. Time to recovery was compared between anesthesia groups. Ketamine plus dexmedetomidine-treated animals recovered significantly faster (mean-rank = 10.33) than those treated with ketamine alone (mean-rank = 26.63) (Figure 2 A) with median recovery times of 12 and 45.5 min, respectively (U = 15, z = -4.655, r = 0.78, $p < 0.005$). Male and female animals showed no significant difference in the time to recovery (U = 36, z = -0.398, r = 0.19, p = 0.730) (Figure 2 B).

Duration of anesthesia and analgesia. Both ketamine and dexmedetomidine provide analgesia in addition to their sedative and anesthetic properties. We randomly selected 6 macaques to be anesthetized with ketamine plus dexmedetomidine to determine the duration of anesthesia and analgesia. Animals were observed for lack of withdrawal to a toe pinch using a hemostat to the first click. None of the 6 animals showed any responsiveness at any time during the 30-min duration of anesthesia. Analgesia was tested for 45 min in the first 2 animals undergoing this procedure. At 45 min, both animals abruptly woke up in response to the toe pinch. Therefore, the remaining animal were tested for only 30 min to avoid injury to personnel.

Discussion

We hypothesized that a high dose of the α2 agonist dexmedetomidine (0.03 mg/kg) in combination with a low dose of ketamine (1.5 mg/kg) and reversal by atipamezole given to rhesus macaques between 4.4 and 12.3 y of age would result in a faster time to recovery than anesthesia induced with 10 mg/kg ketamine alone. Macaques treated with ketamine plus dexmedetomidine and atipamezole recovered in a third of the time required for macaques treated with ketamine alone. The time to recovery was not sex-dependent as male and female macaques recovered after a similar interval. We also found that the use of ketamine plus dexmedetomidine provided 30 min of anesthesia and analgesia for a mild to moderately painful stimulus.

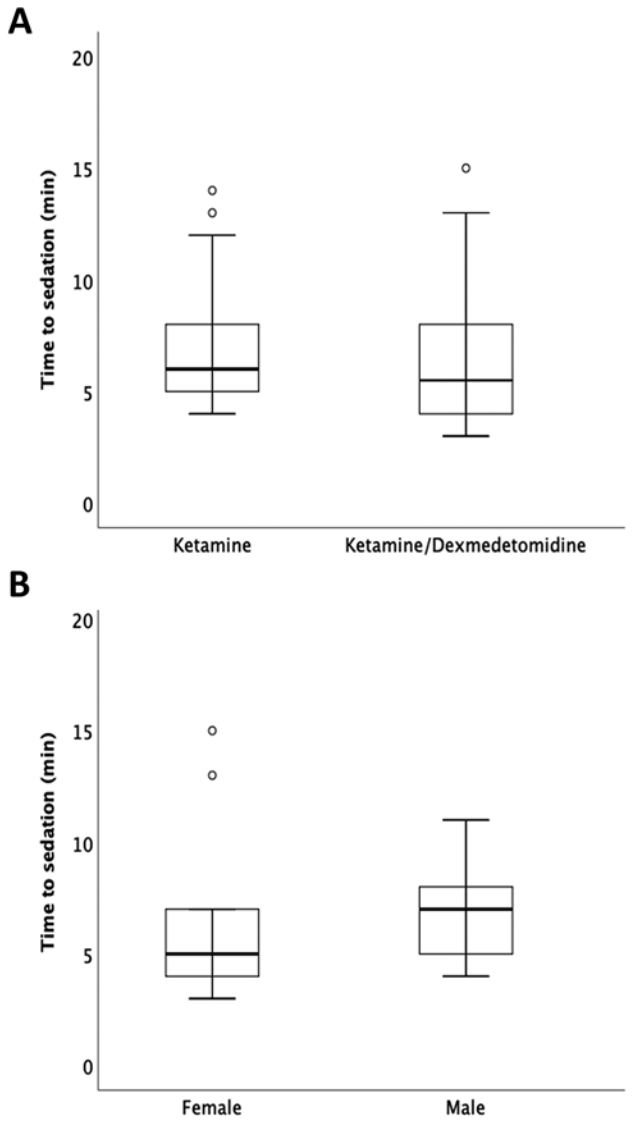

Figure 1. (A) Time to sedation (min) was compared between ketamine and ketamine plus dexmedetomidine-treated rhesus macaques. No significant difference was found between the 2 groups ($P = 0.239$). (B) The impact of sex on median time to sedation was compared between male and female animals. No significant effect of sex was found on median time to sedation ($P = 0.489$). Horizontal black lines within boxes represent the median value. Numbered open circles above boxes represent outlier animals.

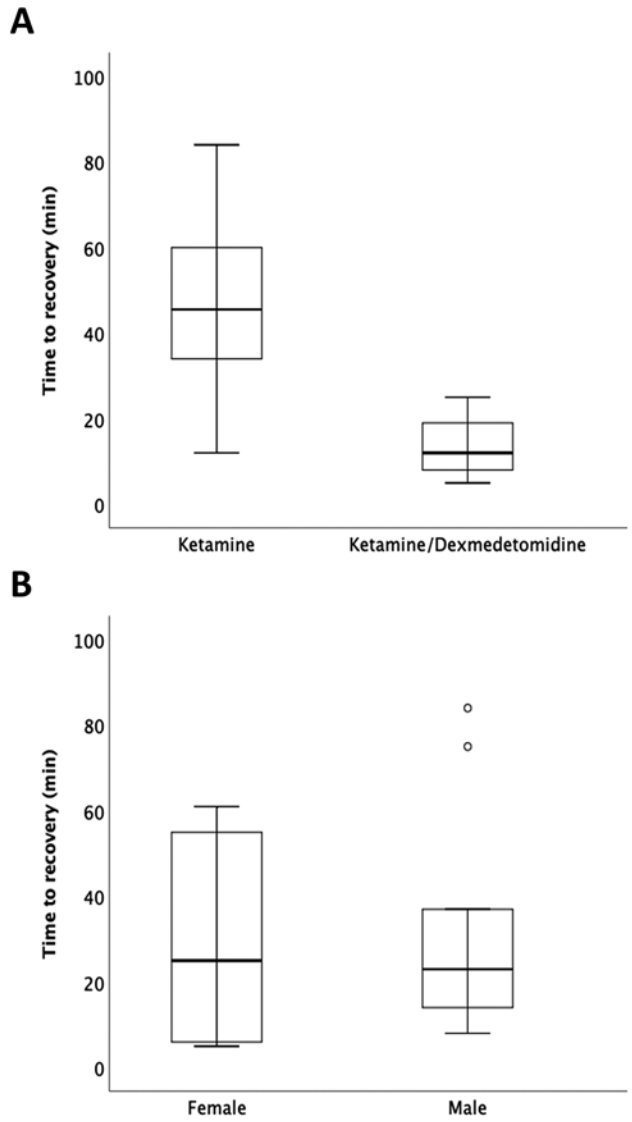

Figure 2. (A) Time to recovery (min) was compared between ketamine and ketamine plus dexmedetomidine-treated rhesus macaques. Macaques treated with ketamine plus dexmedetomidine recovered significantly faster than macaques sedated with ketamine alone ($P < 0.005$). (B) The impact of sex on time to recovery was compared between male and female rhesus macaques. There was no significant effect of sex on median time to recovery ($P = 0.730$). Horizontal black lines within boxes represent the median value. Numbered open circles above boxes represent outlier animals.

Animals treated with α-agonists show side effects of peripheral vasoconstriction, reflex bradycardia, and low peripheral oxygen saturation.[9] Bradycardia, a known side effect of dexmedetomidine, was observed in all macaques treated with ketamine plus dexmedetomidine (heart rate as low as 70 bpm compared with 140 to 180 bpm with ketamine alone). To prevent low oxygen saturation, 100% oxygen supplementation by facemask was provided to all macaques sedated with ketamine plus dexmedetomidine. In macaques that were anesthetized for 30 to 45-min, heart rate and oxygen saturation were monitored every 5 min in addition to the application of a noxious stimulus. At no point did any animal exhibit unexpectedly profound bradycardia or insufficient oxygen saturation. Provision of 100% oxygen by facemask is routinely performed at our institution when using this combination of drugs to prevent low oxygen saturation. We recommend if this combination of drugs is used for procedures of any duration greater than 15 min, then pulse oximetry should be standard and oxygen by facemask be available in case oxygen saturation falls below acceptable levels.

While this study specifically examined the use of a low dose of ketamine combined with a high dose of dexmedetomidine for short procedures, we and others have found that use of other drugs in combination (benzodiazepines, α-agonists, opioids, and/or neuroactive steroids) with or without ketamine provide smoother induction of anesthesia and recovery than ketamine alone, even when the other drugs are administered at low doses.[1] However, the choice of anesthetic agents should always take into consideration the individual animal being anesthetized. The use of α2 agonists requires an understanding of known side effects. Animals that should not receive dexmedetomidine include pregnant animals and those with

heart conditions, as dexmedetomidine can affect fetal blood pressure[11] and conduction within the heart,[7] respectively. In addition, dexmedetomidine is not appropriate in diabetic or hypertensive animals due to the inhibition of insulin secretion[8] and induction of peripheral vasoconstriction,[14] respectively. The use of ketamine also entails risks; for example, neuroapoptosis occurs in neonates occurs after perinatal exposure,[2] and cognitive impairment has been demonstrated.[12]

Others have shown that α-agonists alone are insufficient for restraint in rhesus macaques.[10] However, low dose ketamine combined with xylazine does provide adequate restraint in rhesus macaques.[10] Dexmedetomidine has 40-times greater activity at the α2 receptor than does xylazine.[19] Therefore, we reasoned that substituting dexmedetomidine for xylazine would provide similar restraint and possibly provide a moderate amount of analgesia.

The α2 receptor activity in the dorsal horn of the spinal cord is responsible for the analgesic property of dexmedetomidine.[6,16] However, further definitive attribution of the analgesia to receptor subtypes A, B, and C is difficult, as location and number vary among species.[3,15] We speculate that due to the greater affinity of dexmedetomidine over xylazine for α2 receptors, the analgesia provided by dexmedetomidine would be more robust than that of xylazine. Analgesia afforded by ketamine is dose-dependent; low doses provide more analgesia than do high doses, which instead provide more anesthesia due to changes in receptor binding.[17] Therefore, a low dose of ketamine plus a high dose of dexmedetomidine would likely provide adequate analgesia for short duration, moderately painful procedures. However, atipamezole would reverse the analgesia afforded by dexmedetomidine, perhaps requiring additional postprocedural analgesia.

In conclusion, we have shown that a low dose of ketamine plus a high dose of dexmedetomidine and reversal with atipamezole results in a significantly shorter recover time than does ketamine alone. The time to recovery was not influenced by sex. Furthermore, this combination provided at least 30 min of restraint and analgesia adequate for mild to moderately painful procedures. We have used this combination successfully in our facility for short-duration procedures, including small volume blood collection, intradermal tuberculin placement, transfer into shipping crates, and evaluation and repair procedures for wounds of limited severity. As rhesus macaques are commonly housed in our facilities for over 25 y, we plan this ketamine-sparing drug combination for anesthesia whenever it is appropriate.

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