Butorphanol–Azaperone–Medetomidine for the Immobilization of Rhesus Macaques (Macaca mulatta)

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Butorphanol–Azaperone–Medetomidine for the Immobilization of Rhesus Macaques (*Macaca mulatta*)

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Maximizing animal wellbeing by minimizing drug-related side effects is a key consideration when choosing pharmaceutical agents for chemical restraint in nonhuman primates. One drug combination that may promote this ideology is butorphanol (27.3 mg/mL), azaperone (9.1 mg/mL), and medetomidine (10.9 mg/mL; BAM). Based on results from a pilot study, 2 doses of BAM (16 and 24 µL/kg IM) were compared in healthy, 3-y-old rhesus macaques. Physiologic parameters and anesthetic quality were assessed and recorded every 5 min. Experimental endpoints were established for hypoxemia (85% or less peripheral oxygen saturation with oxygen supplementation), pulse rate (80 bpm or less for 2 consecutive readings), mean arterial pressure (MAP; 50 mm Hg or less), and hypothermia (97 °F or less); if any endpoint was achieved, medetomidine was reversed by using atipamezole (0.22 mg/kg IM). Both BAM doses resulted in immobilization of all animals with no clinically significant differences between groups. All animals initially exhibited hypoxemia that resolved with oxygen supplementation. Regardless of dose, most macaques (71%) reached established experimental endpoints for bradycardia (62 to 80 bpm) or hypotension (44 to 50 mm Hg MAP). Given the results of this study, our recommendation regarding the use of 16- or 24-µL/kg BAM for immobilizing rhesus macaques is dependent on caution regarding cardiopulmonary parameters and the provision of supplemental oxygen.

Abbreviations: BAM, butorphanol–azaperone–medetomidine; MAP, mean arterial pressure

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Maximizing animal wellbeing by minimizing drug-related side effects is a key consideration when selecting pharmaceutical agents for chemical restraint of NHP. The principle of refinement dictates that efforts should be made to reduce animal distress and discomfort during scientific procedures.[47] Investigating the use of novel drugs and drug combinations that have the potential to improve animal wellbeing provides an avenue to discover prospective refinements.

No ideal pharmaceutical agent or agent combination exists that provides optimum immobilization in macaque species without unwanted side effects. This dearth is demonstrated by ketamine hydrochloride—the most common chemical restraint agent used in macaques.[12,19] Advantages of ketamine use include low-cost, potency, effectiveness, and reliability with rapid induction;[38] a wide safety margin and high therapeutic index with few cardiopulmonary effects;[7,11,38] and loss of the bite reflex with intact laryngeal reflexes.[7,38] Despite these advantages, ketamine use can result in pain or irritation due to injection;[6,52] poor muscle relaxation with tonic–clonic movements;[12,52] volume-dependent muscle or nerve damage;[11] ptyalism, dysphoria, delirium, emergence reactions, and tolerance;[7,39,48] as well as decreased feed intake after administration.[20,38,51] In addition, ketamine has variable analgesic properties, and lack of reversibility makes it a suboptimal immobilization agent.[27,38,48,51]

When identifying pharmaceutical agents with the potential to refine current chemical restraint practices in NHP, drugs used in other species should be considered. Developed in 2003 as an alternative to ultrapotent opioids, a combination of butorphanol tartrate (27.3 mg/mL), azaperone tartrate (9.1 mg/mL), and medetomidine hydrochloride (10.9 mg/mL; BAM) has been used for the immobilization of a wide range of wildlife species. These species include white-tailed deer (*Odocoileus virginianus*),[33,34,49] Nubian ibex (*Capra nubiana*),[26] Rocky Mountain elk (*Cervus canadensis nelsoni*),[60] blesbok (*Damaliscus pygorgus phillipsi*),[45] American beavers (*Castor canadensis*),[43] black bears (*Ursus americanus*),[61] and African lions (*Panthera leo*).[46]

In addition, these drugs—butorphanol, an opioid agonist–antagonist with analgesic (variable), antitussive, and antiemetic properties; azaperone, a butyrophenone tranquilizer with antipsychotic, sedative, and antiemetic properties; and medetomidine, a potent α_2 adrenergic agonist sedative with analgesic, anxiolytic, and muscle-relaxant properties— are used individually in a wide variety of animal species.[8,12,13,31,38,47] Of these agents, medetomidine is the only drug able to solely produce recumbency and serves as the driver for immobilization.[13,61] In combination, these drugs have the benefits of small injection volumes, antiemesis, smooth induction and recovery, reversibility, mild soft tissue analgesia, muscle relaxation, and lack of ptyalism. Reported side effects of BAM include nonlife-threatening cyanosis, hypoxemia, bradycardia, bradypnea, as well as rare, mild twitching and muscle tremors.[26,33,34,43,45,46,49,60,61]

Given the immobilization success and minimal health effects reported for other species, the goal of the current study was to

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explore the use of BAM in rhesus macaques (*Macaca mulatta*). The objectives were to 1) establish a minimal dose that achieves 45 min of chemical immobilization and maintains physiologic values according to predefined thresholds; 2) characterize dose-dependent changes in physiologic values and anesthetic depth over time; 3) assess induction and recovery quality and duration; and 4) document side effects. We hypothesized that BAM at a minimum of 16 µL/kg IM would provide 45 min of chemical immobilization of rhesus macaques while maintaining acceptable physiologic values.

Materials and Methods

Humane care and use of animals. All procedures were approved by the IACUC of the University of Florida and The Mannheimer Foundation and were performed at The Mannheimer Foundation, an AAALAC-accredited facility. Macaques were maintained according to the Animal Welfare Act[1] and Regulations[2] and the *Guide for the Care and Use of Laboratory Animals*.[22]

Animals. Female (n = 17; weight [mean ± 1 SD], 4.96 ± 0.30 kg) and male (n = 17; weight, 5.42 ± 0.42 kg) juvenile rhesus macaques (*Macaca mulatta*; age, 2.75 ± 0.07 y) were used for this study. Animals were individually caught in nets, boxed, and transferred (without sedation) from outdoor, same-sex social housing units. Subjects were singly indoor-housed in squeeze-back cages (floor area, 0.4 m^2; height, 76.2 cm) located in a climate-controlled room (65.1 to 80.2 °F [18.3 to 26.8 °C]; relative humidity, 36% to 88%) on a 12:12-h light:dark cycle within visual and auditory contact of conspecifics for a maximum of 7 d. On study completion, macaques were boxed and returned (without sedation) to their outdoor enclosures.

Animals were fed a standard commercial primate diet (5LB2, Lab Diet, St Louis, MO) twice daily and watered free-choice through an automatic system. All macaques were provided with the same manipulanda (mirror, treat ball, plastic dumbbell) and fresh forage daily (popcorn, orange slices, FiberBites [ClearH_20, Westbrook, ME]). After a 3-d minimal acclimation period, macaques were feed-fasted overnight (approximately 12 h) prior to administration of BAM (Wildlife Pharmaceuticals, Windsor, CO).

Animals were SPF (serologically negative) for *Macacine herpesvirus 1*, *Simian retrovirus 1*, *Simian T-lymphotrophic virus 1*, and *Simian immunodeficiency virus*. Macaques were healthy and tuberculosis-free as determined by semiannual physical examinations and intradermal tuberculin tests (mammalian, human isolates; 10 µL; Synbiotics, San Diego, CA). Animals were routinely vaccinated against *Clostridium tetani* (tetanus toxoid; 0.5 mL IM every 5 y; Zoetis, Kalamazoo, MI), *Measles morbillivirus* (0.5 mL SC every 6 mo; M-M-R_{II}, Merck, Whitehouse Station, NJ), and *Rabies lyssavirus* (1 mL SC every 3 y; Rabvac 3, Elanco US, Fort Dodge, IA) and were dewormed with ivermectin (0.2 mg/kg IM every 6 mo; Vetrimec 1%, MWI Animal Health, Boise, ID).

Study design. To prevent confounding factors due to the use of other immobilization agents, net-caught macaques were boxed and transported without sedation from outdoor to indoor enclosures. Animals were initially weighed in transport boxes to determine BAM doses; these indirect body weights were calculated by subtracting the weight of the transport box from the weight of the transport box containing an animal. Direct body weights were collected 3 to 5 d later, during BAM immobilization.

A randomized block design was applied; macaques were grouped by sex before subjects were assigned to dose groups by using a random sequence generator (http://www.random.org/sequences). All immobilization procedures and physiologic monitoring were performed between 0730 and 1200.

Pilot study. Ten macaques (5 female, 5 male) each received 1 of 5 doses of BAM (4, 8, 16, 24, or 32 µL/kg IM). For safety, dose groups (n = 2; 1 female, 1 male) were evaluated stepwise from the lowest to the highest dose. The 4-µL/kg BAM dose was selected to reflect published ranges for medetomidine.[3,7-10,21,27,48,52] Three doses (4, 8, and 16 µL/kg) were diluted 1:5 with sterile bacteriostatic water to ensure an appreciable injection volume. The concentrations of the individual BAM components for each dose are listed in Table 1.

Dose characterization. According to results from the pilot study, 2 doses of BAM were selected for further characterization: 16 µL/kg IM (n = 12; 6 female, 6 male) and 24 µL/kg IM (n = 12; 6 female, 6 male). The 16-µL/kg dose was not diluted as in the pilot study. The person monitoring and recording physiologic values and anesthetic depth was blinded to the BAM dose administered.

BAM administration and health assessment. Each BAM dose was injected into the right or left quadriceps muscle as the cage squeeze-back mechanism was used. On loss of the righting reflex, the animal was transferred to an adjacent procedure room and placed in left lateral recumbency. A physical examination was performed, direct body weight was obtained, hydration status was assessed, and a health score according to the Physical Classification Status of the American Society of Anesthesiologists (scale, 1 to 5) was assigned.[15] For medetomidine reversal, atipamezole (0.22 mg/kg) was injected into the contralateral quadriceps muscle; the macaque was returned to the cage in lateral recumbency and monitored until the animal was sitting upright. Observation continued intermittently for a minimum of 4 h after recovery. A butorphanol antagonist was not administered to mimic clinical conditions that would benefit from mild analgesia after recovery from immobilization.

Physiologic monitoring. Once each macaque was immobilized, physiologic parameters were recorded every 5 min for 45 min. Mucous membrane color and capillary refill time (in seconds) were recorded after direct observation and blanching from digital pressure on the gingival mucosa. A pulse oximeter probe (Masimo SET Rad-5, Masimo, Irvine, CA) was placed on the right cheek to monitor S_pO_2 and pulse rate. Indirect mean arterial pressure (MAP; right upper arm; neonatal cuff size 4 or 5, Unicuff, Frontier Medical Products, Grafton, WI) and rectal temperature were recorded by using a multichannel monitor (nCompass 8100H Series, Criticare Technologies, North Kingstown, RI). Respiratory rate was recorded from manual counts.

Anesthetic quality, depth, and duration. The induction quality (score, 1 to 3) was determined according to the amount of movement and resistance displayed by the animals from BAM administration until immobilization. Anesthetic depth parameters were scored every 5 min for 45 min after immobilization, concurrently with physiologic parameters. The pedal reflex (score, 1 to 5) was assessed by pinching a toe with a pair of hemostats until the first notch locked. Spontaneous movements (for example, muscle twitching, body movements; score, 1 to 5) were assessed through direct observation of the animal. The degree of immobilization (that is, muscle tone; score, 1 to 5) was assessed by allowing the leg to drop from a raised position into the hand of the observer. The depth-of-anesthesia parameters used in this study were based on those developed previously.[27] The recovery quality (score, 1 to 3) was determined according to the amount of movement and resistance displayed by the macaques, from atipamezole administration until the animal could sit upright. Descriptions of each scoring system used to assess anesthetic quality and depth are provided in Figure 1.

Table 1. Doses (µL/kg) of the individual components of the butorphanol–azaperone–medetomidine (BAM) by volume

	Component (mg/kg)		
BAM dose	Butorphanol	Azaperone	Medetomidine
4	0.11	0.04	0.04
8	0.22	0.07	0.09
16	0.44	0.15	0.17
24	0.66	0.22	0.26
32	0.87	0.29	0.35

Anesthetic duration was determined by timing events in minutes. Induction time was measured from the time of BAM injection until the animal was recumbent and could be removed safely from the cage. Time to first recording was measured from the time of BAM injection until the time when the first physiologic recording was taken. Immobilization time was measured from the time of loss of righting reflex until atipamezole administration. Recovery time was measured from the time of atipamezole administration until the time the animal could sit upright. The total anesthetic time was measured from the time of BAM administration until the time the animal could sit upright. A schematic diagram illustrating these parameters and the actions performed at specific time points during BAM administration, monitoring, and recovery is provided in Figure 2.

Interventions and endpoints. During physiologic monitoring, humane interventions included: 1) providing lactated ringers (10 mL/kg SC) for mild dehydration (less than 5%); 2) using supplemental oxygen (flow-by; 1.0 to 1.5 L/min) when S_pO_2 was less than 90%; 3) active warming (warm-water blanket) for rectal temperatures less than 97 °F; and 4) holding an animal with its head in a downward position, with verification of airway patency, when regurgitation occurred. Experimental endpoints included: 1) hypoxemia (S_pO_2 85% or less with oxygen supplementation); 2) bradycardia (80 bpm or less for 2 consecutive readings); 3) hypotension (MAP of 50 mm Hg or less during a single oscillometric measurement); and 4) regurgitation (2 or more episodes during a single immobilization event). When an animal reached an experimental endpoint, immobilization was immediately reversed by using atipamezole, and the macaque was returned to its cage and monitored until it was able to sit upright. Endpoint criteria reflected published reference ranges for macaques.[3,9]

Statistical analysis. Multivariate linear mixed-effects models (lme) that included a normally distributed random intercept (mean = 0, variance = σ^2) for the subject were used to analyze relationships between variables (BAM dose, sex, body weight, S_pO_2, pulse rate, MAP, rectal temperature, respiratory rate, and recording time). Rectal temperature was further explored by using general additive mixed models. General linear models were used to analyze anesthetic duration. Spontaneous movement scores were reclassified as binary variables (that is, present or absent) for analysis. A risk analysis was performed by using binomial generalized linear mixed models to examine the probabilities of an animal experiencing bradycardia, hypotension, spontaneous movement, waking early, or reaching an experimental endpoint.

For all analyses, optimal models were obtained through backward stepwise model selection based on the results of an F-test (linear mixed-effects, general additive mixed, and general linear models) or z-test (general linear mixed models). Each *P* value reported for a nonsignificant variable was obtained from the step prior to the removal of that variable. A *P* value of less than 0.05 was considered significant. All analyses were performed by using R (http://www.R-project.org/). The packages 'nlme',[37] 'lme4',[4] 'mgcv,'[62] and 'MASS'[56] were used for these analyses.

Results

Animal health. All macaques were unremarkable on physical examination, euhydrated, and received a score of 1 (normal healthy patient) according to the Physical Classification Status of the American Society of Anesthesiologists.[15] The mean difference between indirect and direct body weights was 2.2% and ranged from –6.3% to 4.1%.

Pilot study. Induction quality was consistently smooth with little movement among dose groups that received at least 16 µL/kg BAM. The 4- and 8-µL/kg BAM doses did not result in immobilization. These macaques could be safely removed from the cage, but the level of sedation achieved did not allow for use of physiologic monitoring equipment. The 16-µL/kg BAM dose resulted in 27 min of immobilization before 1 macaque recovered and 29 min before the other reached the experimental endpoint for bradycardia. The 24-µL/kg BAM dose resulted in 12 min of immobilization before 1 animal recovered and 33 min before the other reached the endpoint for bradycardia. The 32-µL/kg BAM dose resulted in 13 and 17 min of immobilization before both animals reached experimental endpoints for bradycardia. Initially, 67.7% of macaques monitored were hypoxemic until supplemental oxygen was provided. No other interventions or experimental endpoints were reached during physiologic monitoring. Recovery quality was consistently smooth with little movement among dose groups that received at least 16 µL/kg BAM. The results from the pilot study are summarized in Table 2.

Physiologic parameters. Mucous membranes were pink and capillary refill time was less than 2 s for all time points. All macaques were significantly hypoxemic (S_pO_2, 4% to 76%) at the first physiologic reading and received supplemental oxygen, after which they maintained normoxia (S_pO_2, greater than 89%). There were no significant differences between doses ($P = 0.1471$) or sexes ($P = 0.1035$). Pulse rate significantly ($P < 0.0001$) decreased over time by 0.71 bpm/min (Figure 3 A), and there were no significant differences between doses ($P = 0.0582$) or sexes ($P = 0.5087$). Pulse rate was negatively correlated ($P < 0.0001$) with rectal temperature, increasing by approximately 6.36 bpm for each 1 °F decrease (Figure 3 B). MAP significantly ($P < 0.0001$) decreased over time by approximately 0.33 mm Hg/min (Figure 3 C), and there were no significant differences between doses ($P = 0.3105$) or sexes ($P = 0.2775$). Rectal temperatures changed significantly ($P < 0.0001$) over time. Mean rectal temperature increased 0.05 °F/min during the first 2 recording intervals (11.2 min) and then decreased 0.05 °F/min. The 24-µL/kg BAM dose resulted in a significantly ($P = 0.0381$) higher (by 0.39 °F) rectal temperature compared with the 16-µL/kg BAM dose (Figure 3 D); there was no significant difference between sexes ($P = 0.6036$). Rectal temperature was negatively correlated ($P = 0.0154$) with respiratory rate, decreasing approximately 0.01 °F for each 1 breath per minute increase (Figure 3 E). Respiratory rate significantly ($P < 0.0001$) decreased over time by 0.22 breaths per minute for each 1 min (Figure 3 F), and there were no significant differences between doses ($P = 0.8531$) or sexes ($P = 0.6308$). Respiratory rate was negatively correlated ($P = 0.0004$) with rectal temperature, increasing by approximately 3.5 breaths per minute for every 1 °F decrease (Figure 3 G). Respiratory rate was positively correlated ($P = 0.0168$) with MAP, increasing 0.09 breath per minute for each 1 mm Hg (Figure 3 H).

Anesthetic quality, depth, and duration. Induction quality was always smooth, with little movement, and independent of dose

Score	Quality of Induction	Pedal Reflex	Spontaneous Movements	Immobilization	Recovery Quality
1	Smooth, little movement	No response	No movement	Muscles flaccid and relaxed	Smooth, little movement
2	Moderate	Delayed, weakly pulls away	Twitching of hands or feet	Mild muscle tone	Moderate
3	Rough, lots of movement	Immediate, weakly pulls away	Facial movements	Moderate muscle tone	Rough, lots of movement
4		Flexes or extends digits	Limb movements	Normal or exaggerated muscle tone	
5		Exaggerated, strongly pulls away	Whole body movement	Was never immobilized	

Figure 1. Score chart used to gauge anesthetic quality and depth after administration of butorphanol–azaperone–medetomidine intramuscularly. Descriptions of each score are provided for the 5 parameters assessed. Induction and recovery quality were scored on a scale of 1 to 3 and assessed once, whereas scores for pedal reflex, spontaneous movements, and immobilization ranged from 1 to 5 and were assessed every 5 min for the duration of immobilization.

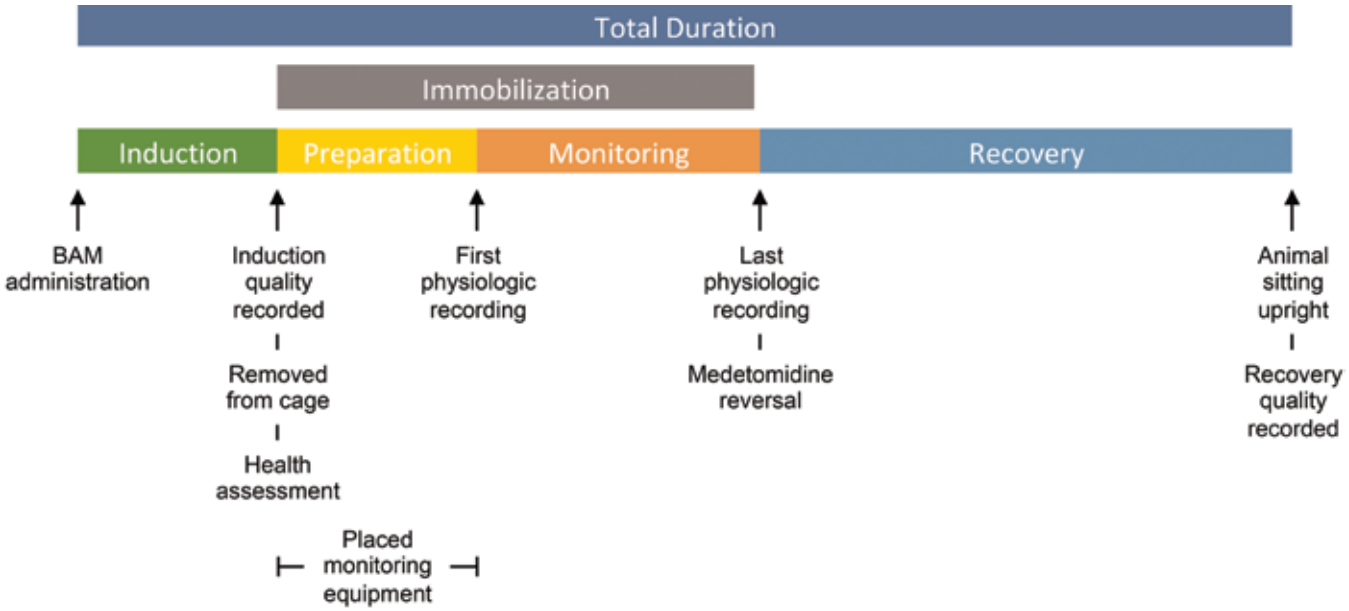

Figure 2. Schematic of butorphanol–azaperone–medetomidine (BAM) administration, monitoring, and recovery. Times are represented by the shaded bars, with the actions performed at specific time-points indicated beneath the bars.

or sex. The depth of anesthesia scores were consistent between doses and sexes over time. Pedal reflex assessment always resulted in no response, except for 1 macaque that had a single delayed response and weakly pulled away at 25 min after BAM administration. Overall, spontaneous movements were predominantly twitching of the hands and feet (37.5%) but ranged from no movement (25.0%) to whole-body movements (4.1%). Spontaneous movements significantly ($P = 0.0111$) decreased over time (Figure 4), and there were no significant differences between doses ($P = 0.7647$) or sexes ($P = 0.3393$). Overall, immobilization scores primarily consisted of flaccid and relaxed muscles (91.7%), with a few animals exhibiting mild, moderate

Table 2. Pilot study results (n = 10) for butorphanol-azaperone-medetomidine (BAM) by dose (µL/kg)

		Endpoint criterion met?				
BAM dose	Sex	Hypoxemia	Bradycardia	Hypotension	Immobilization time (min)	BAM injection volume (mL)
4	Male	—	—	—	0	0.09[a]
4	Female	—	—	—	0	0.10[a]
8	Male	—	—	—	0	0.18[a]
8	Female	—	—	—	0	0.18[a]
16	Male	Yes	Yes	No	29	0.38[a]
16	Female	Yes	No	No	27	0.40[a]
24	Male	Yes	No	No	33	0.12
24	Female	No	Yes	No	12	0.10
32	Male	No	Yes	No	17	0.16
32	Female	Yes	Yes	No	13	0.15

Supplemental oxygen (flow-by; 1.0–1.5 L/min) was provided when S_pO_2 ≤90%. Endpoint criteria were applied for hypoxemia (S_pO_2 ≤85% despite supplemental oxygen), bradycardia (≤80 bpm for 2 consecutive readings), and hypotension (mean arterial pressure, ≤50 mm Hg). The 4- and 8-µL/kg BAM doses did not provide sufficient immobilization for the use of physiologic monitoring equipment.
[a]BAM diluted 1:5 in bacteriostatic water

or normal muscle tone (8.3%). Recovery quality was always smooth, with little movement and independent of dose or sex.

There were no significant differences in anesthetic duration between doses ($P \geq 0.2098$) or sexes ($P \geq 0.2867$). For all subjects, the induction time (mean ± 1 SD) was 4.0 ± 1.2 min, time to first recording was 7.4 ± 1.2 min, immobilization time was 26.3 ± 15.7 min, recovery time was 11.9 ± 9.1 min, and total anesthetic time was 42.1 ± 13.1 min. Three macaques (12%) recovered prior to atipamezole administration. The probability of an animal recovering from BAM significantly increased over time (odds ratio = 1.9; $P < 0.0001$).

Interventions and endpoints. Macaques remained euhydrated and did not require fluid therapy. After the first monitoring time point, supplemental oxygen was required for all subjects. All rectal temperatures were higher than 97 °F, and active warming was unnecessary. No regurgitation occurred. The probability of an animal reaching an experimental endpoint was not affected by dose ($P \geq 0.9640$) or sex ($P \geq 0.1601$). After 25 min of monitoring, 12 (50%) of the 24 macaques had reached the experimental endpoint for bradycardia (62 to 80 bpm; Figure 3 A). The probability of an animal experiencing bradycardia significantly increased over time (odds ratio = 3; $P < 0.0001$). None of the factors tested affected the likelihood of an animal experiencing hypotension. Overall, the majority (17 of 24) of macaques achieved experimental endpoints for bradycardia or hypotension or both. In addition to the 12 animals that were solely bradycardic, 4 animals (17%) became hypotensive (MAP, 44 to 50 mm Hg) and 1 (4%) became both bradycardic (65 and 66 bpm) and hypotensive (MAP, 46 mm Hg). Whereas another 3 macaques recovered before atipimezole administration, the remaining 4 (17%) achieved the objective of 45 min of monitoring. A risk analysis demonstrated no evidence that either dose increased the risk of reaching an experimental endpoint.

Discussion

Investigating the use of novel pharmaceutical agents for immobilization is an important approach to refinement as a method of mitigating distress and discomfort during routine veterinary or scientific procedures. This study exemplified this concept by exploring the potential use of BAM for immobilization of rhesus macaques. Results demonstrated that BAM doses of 16 and 24 µL/kg IM had the advantages of immobilization for at least 25 min, with small injection volumes (less than 0.15 mL), smooth and rapid induction, absence of pedal reflex, primary agent reversibility, and smooth recovery. Disadvantages of BAM in this study included severe but reversible initial hypoxemia (prior to oxygen supplementation) and cardiovascular effects (bradycardia and hypotension) with mild-to-moderate spontaneous movements during immobilization. These findings, with the exception of hypotension, were consistent with those reported in previous studies.[26,33,34,43,45,46,49,60,61]

Individual doses of butorphanol and medetomidine for the 16- and 24-µL/kg BAM doses were higher than published recommendations for sole agent and multiagent administration (butorphanol, 0.05 to 0.2 mg/kg IM; medetomidine, 0.01 to 0.2 mg/kg IM).[3,7-9,21,27,48,52] The doses administered in the current study were more than 4 times the recommended dose of butorphanol and twice that of medetomidine. In the pilot study, the lowest BAM dose (4 µL/kg IM) was based on the recommended dose for medetomidine[3,7-10,21,27,48,52] and increased incrementally to higher doses suggested by the manufacturer for similarly sized nondomestic species (0.1 mL), such as fishers (*Pekania pennanti*; body weight, 2.0 to 6.0 kg) and groundhogs (*Marmota monax*; body weight, 2.2 to 5.5 kg).[59]

Unexpectedly, the 4- and 8-µL/kg BAM doses did not adequately immobilize the macaques to allow for physiologic monitoring. We had anticipated that this drug combination would result in additive effects to reduce individual drug doses, thereby reducing side effects; however, the individual drug doses required for immobilization were greater, and side effects (hypoxemia, bradycardia) were not reduced. This result potentially is due to the drug formulation, which may not contain the optimal drug ratios for rhesus macaques.

Direct body weights were 0.18 ± 0.22 kg less than initial, indirect body weights. These slight changes in body weight are likely due to minor stresses associated with changes in the environment and housing conditions (from outdoor, group housing to indoor, single housing). To avoid potentially confounding effects from other sedatives, we based the BAM doses on indi-

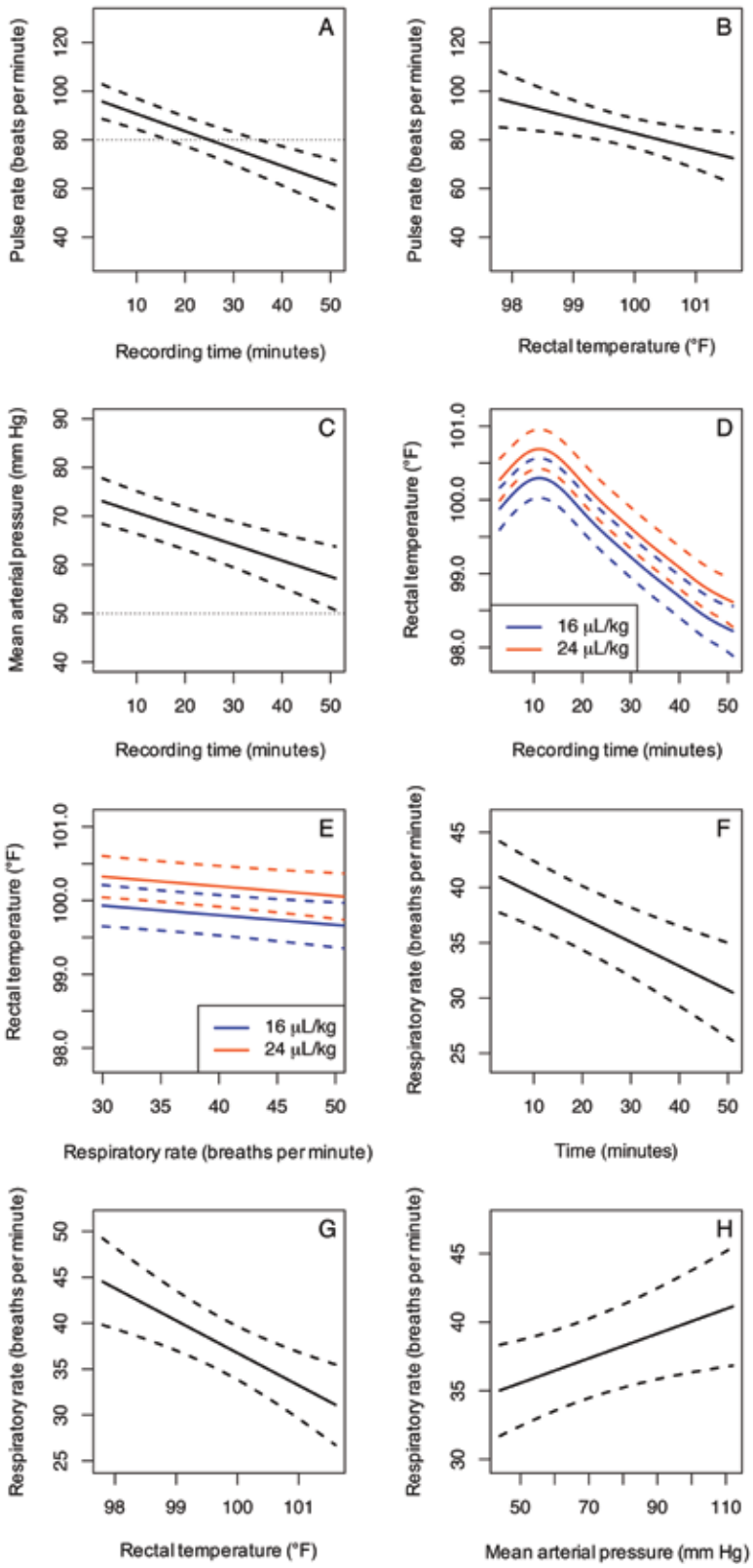

Figure 3. Physiologic parameters were recorded every 5 min after the intramuscular administration of 16- or 24-µL/kg butorphanol–azaperone–medetomidine, recumbency, and application of physiologic monitoring equipment. (A) Pulse rate (beats per minute) significantly ($P < 0.0001$) decreased over time (in minutes) and (B) significantly ($P < 0.0001$) decreased as rectal temperature (°F) increased. (C) Mean arterial pressure (mm Hg) significantly decreased over time ($P < 0.0001$). (D) Rectal temperature significantly changed over time ($P < 0.0001$); although significantly higher at the 24-µL/kg dose ($P = 0.0381$), the difference (0.39 °F) was not clinically relevant. (E) Rectal temperature significantly decreased as respiratory rate (breaths per minute) increased ($P = 0.0154$). (F) Respiratory rate significantly decreased over time ($P < 0.0001$) and (G) significantly decreased as rectal temperature increased ($P = 0.0004$). (H) Respiratory rate significantly increased as mean arterial pressure increased ($P = 0.0168$). For all figures, the solid line represents the population line of best fit from the optimal model, dashed lines represent the 95% CI, and dotted lines represent the experimental endpoint. Lines of best fit for each explanatory variable were obtained by constraining other significant explanatory covariates to their mean values.

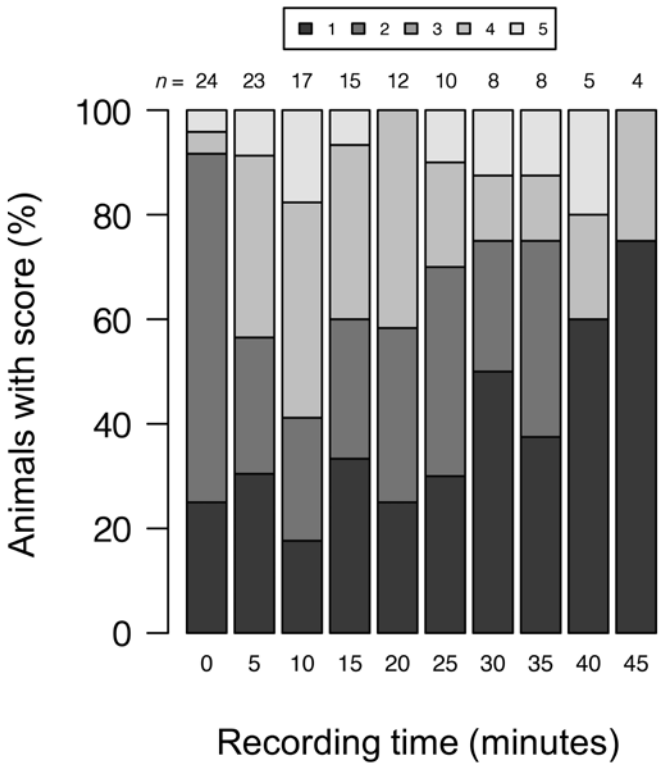

Figure 4. Percentage of animals exhibiting spontaneous movement over time. Scores were recorded every 5 min after intramuscular administration of 16- or 24-µL/kg butorphanol-azaperone-medetomidine and categorized as no movement (1); twitching of hands and feet (2); facial movements (3); limb movements (4); or whole-body movements (5). The number of animals scored at each time point (*n*) is displayed above each column as some animals woke before 45 min of monitoring. Overall, spontaneous movements significantly decreased over time ($P = 0.0111$).

rect body weights of unsedated animals. As a consequence, 9 subjects received doses 0.01 mL less (1 subject), 0.01 mL greater (7 subjects), or 0.02 mL greater (1 subject) than they would have had their body weights been measured directly. However, given that disparities between indirect and direct body weight resulted in such small differences in drug dose, they are unlikely to have altered study results.

The initial hypoxemia observed prior to oxygen supplementation is consistent with results reported in prior BAM studies involving white-tailed deer,[33,34,49] Rocky Mountain elk,[60] blesbok,[45] and beavers.[43] In addition to the lack of oxygen supplementation, 2 key factors likely contributed to the low S_pO_2 values obtained during the initial readings in the current study: peripheral vasoconstriction and the use of pulse oximetry. Vasoconstriction is attributed to the agonistic effects of medetomidine on peripheral α_2-adrenergic receptors in vascular smooth muscle, resulting in reduced tissue perfusion.[9,20,38,50,55,57] Although pulse oximetry is commonly used during routine anesthetic procedures due to its simplicity and noninvasiveness, this modality is known to be inaccurate in cases of poor tissue perfusion (for example, peripheral vasoconstriction, hypotension, hypothermia).[23] In addition, the use of pulse oximetry to measure hypoxemia is poorly sensitive compared with arterial blood gas measurement. Factors contributing to this poor sensitivity include tissue thickness, skin pigment, poor pulsatile blood flow, spontaneous movement, and machine characteristics.[16,20,32] The abnormally low S_pO_2 readings that we observed initially were likely due to a combination of peripheral vasoconstriction secondary to medetomidine administration and compounded by the use of pulse oximetry.[20,23,53]

Moreover, medetomidine causes centrally mediated bradycardia and hypotension due to inhibition of sympathetic tone as a result α_2-adrenoreceptor agonism, largely in the locus coeruleus of the brain stem.[38,50,55,57] The bradycardia in our study was not life-threatening despite the significant decrease in pulse rate over time; the lowest pulse rate detected was 62 bpm, with the majority of the measurements below the experimental endpoint (80 bpm or less over 5 min) ranging from 65 to 79 bpm. According to the statistical model, the mean predicted value pulse rate after 50 min of immobilization is approximately 50 bpm. Of the subjects that reached the experimental endpoint for bradycardia, all except for 1 maintained normotension according to study standards (greater than50 mm Hg). These results are consistent with previous studies using BAM, in which mild-to-moderate bradycardia occurred in white-tailed deer,[33] blesbok,[45]and African lions[46] and when intravenous medetomidine was administered to rhesus macaques.[9] Similar to detecting hypoxemia with pulse oximetry, the detection of pulse rate can be affected by poor peripheral perfusion and decreased peripheral arterial pulsation (the signal used by the pulse oximeter to estimate heart rate) caused by vasoconstriction and hypotension. In addition, these factors increase the chance of motion artifacts that may occur with spontaneous movement during immobilization.[16,32]

The hypotension that occurred in 5 macaques in our study was previously reported in blesbok; however, only 4 of the 9 cited BAM studies assessed blood pressure.[26,33,34,43,45,46,49,60,61] The significant decrease in MAP over time was expected, given the high doses of medetomidine administered, because hypotension is a reported side effect of this drug.[9,38,50] Interestingly, although there are no reports of azaperone use in macaques, this drug may have contributed to the cardiovascular effects observed during our study. When administered intramuscularly in domestic pigs (*Sus domesticus*) and horses (*Equus ferus caballus*), side effects include mild bradycardia and hypotension induced by α_1-adrenergic blockade.[13,14,28,38,47,58] Furthermore, noninvasive blood pressure measurements have been reported to read significantly lower MAP compared with 'gold standard' invasive arterial blood pressure, particularly in hypotensive patients with MAP measurements lower than 65 mm Hg. Nonetheless, these studies support the use of oscillometric MAP as the best alternative to invasive monitoring and for simply determining if a patient is hyper- or hypotensive.[17,25,29,41] The hypotensive subjects in our study might have had a higher MAP value if measured invasively.

The rectal temperatures that we observed (99.85 ± 0.82 °F) changed minimally throughout the study. Because thermal support was not provided unless rectal temperature was lower than 97 °F, the steady mild decrease (that followed a small increase for 11.2 min) was anticipated in light of medetomidine-induced CNS depression and decreased muscular activity during immobilization. These temperature changes are consistent with BAM studies in wildlife species[26,33,34,46,49,61] and medetomidine studies in domestic dogs (*Canis familiaris*)[36,40] and can be attributed to a combination of factors, including peripheral vasoconstriction (reducing radiative heat loss), central redistribution of blood, and reduced metabolic rate.[36,50,55] Although occasional muscle twitching and tremors occurred in our macaques, they were unlikely to contribute to changes in rectal temperature as would normal muscular activity. According to statistical analysis, rectal temperature was significantly higher (0.39 °F) for the 24-µL/kg dose compared with the 16-µL/kg BAM dose, but this difference was not clinically relevant. Similarly, the statistically significant

negative correlations between rectal temperature and respiratory rate were not clinically relevant and can be explained by inconsequential evaporative respiratory heat loss.

The significant decrease in respiratory rate over time was most likely due to both the combination of medetomidine and butorphanol as well as the magnitude of the dose received. Butorphanol as a sole agent is known to cause respiratory depression,[13,38] whereas there is minimal respiratory depression associated with medetomidine alone. However, studies in domestic dogs[24,40] and cats (*Felis catus*)[44] determined that medetomidine, or its active isomer dexmedetomidine, in combination with butorphanol or other opioids significantly reduces respiratory rate.[50] The positive correlation between respiratory rate and MAP that we noted in our macaques was not clinically relevant, given that the rate of change (0.09 bpm per 1 mm Hg) was minimal.

The pedal reflex was absent during immobilization, albeit subjects demonstrated sporadic, infrequent spontaneous movements. Most movements included twitching of the hands and feet, but several animals exhibited whole-limb (5 of 24 subjects) or whole-body movements (1 of 24 subjects). Movement was previously noted after intravenous medetomidine administration, during which macaques displayed mild and transient muscle tremors.[9] Previous BAM studies in white-tailed deer[26,33] and African lions[46] reported muscle tremors and leg movements. In addition, 3 subjects recovered before atipamezole was administered and spontaneously attempted to rise at 23 (1 subject) and 40 (2 subjects) min after BAM administration. Although auditory stimuli were not evaluated during this study, other studies in macaques have demonstrated an arousal response to sound while sedated with medetomidine.[9,35]

Although recovery time did not differ between groups, note that the same dose of atipamezole (0.22 mg/kg) was administered regardless of the magnitude of the BAM dose. The atipamezole dose we used is recommended for reversal of a 0.04-mg/kg dose of medetomidine (that is, 4-µL/kg BAM dose). In future studies, a dose appropriate for the medetomidine dose administered should be given.

Given the ideal health status and body condition of the juveniles used in this study, future investigation into the use of BAM in rhesus macaques should include characterization of physiologic responses, depth, and quality of anesthesia, and anesthetic duration in other age groups, body conditions, and health states, to account for differences in drug distribution, metabolism, and excretion. Because all 3 drug components in BAM are lipophilic and metabolized by hepatic glucuronidation,[18,42,50] factors that affect body fat composition or compromise liver function have the potential to influence the effects of BAM. Drug metabolism and distribution are altered by increased fat stores and obesity, which represent confounding variables in drug trials.[5] In addition, obesity and increasing age are typically accompanied by comorbidities, such as metabolic, renal, and coronary disease, that may affect how drugs are distributed, metabolized, and eliminated.[5] Furthermore, geographic origin, age, sex, and some disease states can alter glucuronidation.[30]

Based on the results of the current study, BAM is a potential drug combination for short-term immobilization of rhesus macaques. Lower BAM doses (for example, 8 µL/kg IM) may provide adequate sedation for short procedures, such as relocation or obtaining body weight. Combining BAM with other drugs should be examined for procedures requiring longer immobilization times, greater pain reduction, or a deeper plane of anesthesia. Caution during its use is advised in light of its cardiopulmonary effects, specifically hypoxemia and bradycardia. A minimum of supportive care comprising supplemental oxygen, thermal support, and pulse-rate monitoring are recommended during BAM use in rhesus macaques. Further studies should assess the safety and efficacy of BAM in rhesus macaques.

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