Effects of ketamine and magnesium on the minimum alveolar concentration of isoflurane in goats

Patricia Queiroz-Castro, DVM, MSc; Christine Egger, DVM, MVSc; Marcia A. Redua, DVM, MSc; Barton W. Rohrbach, VMD, MPH; Sherry Cox, PhD; Tom Doherty, MVB, MSc

MINIMUM AVEOLAR CONCENTRATION IS DEFINED AS THE END-TIDAL CONCENTRATION OF A VOLATILE ANESTHETIC THAT PREVENTS PURPOSEFUL MOVEMENT IN RESPONSE TO A NOXIOUS STIMULUS IN 50% OF THE POPULATION.1 Because volatile anesthetics dose-dependently depress the cardiovascular and respiratory systems, any reduction in the MAC of volatile anesthetics by administration of analgesics or sedatives may improve cardiovascular and respiratory function, while providing additional comfort in the postoperative period.2

Ketamine is an anesthetic drug with analgesic properties. It is a noncompetitive antagonist of NMDA receptors and reduces the ISOMAC in dogs3,4 and goats,5 MAC of enflurane in dogs,6 and MAC of halothane in rats7 and horses.8

Magnesium is a noncompetitive antagonist at NMDA receptors that reduces MAC of halothane in a dose-dependent fashion when administered to rats.9 In addition, magnesium potentiates the actions of volatile anesthetics by interactingadditively at NMDA receptors in vitro.10

The potency of volatile anesthetics is increased by noncompetitive NMDA antagonists.11 Results of in vitro pharmacologic studies12,13 by use of Xenopus oocytes indicate that ketamine, magnesium, and volatile anesthetics interact at NMDA receptors, suggesting that the hypnotic actions of magnesium and ketamine are synergistic and that the analgesic effects of magnesium and ketamine are likely to be enhanced in the presence of volatile anesthetics.

The purpose of the study reported here was to determine the effects of ketamine, magnesium sulfate, and their combination on ISOMAC in goats. It was hypothesized that ketamine and magnesium sulfate would decrease ISOMAC and that the combination of ketamine and magnesium sulfate would have an additive effect in reducing ISOMAC.

Materials and Methods

Goats—The study was approved by the University of Tennessee, Animal Care and Use Committee. Eight healthy adult castrated male mixed-breed goats, weighing from 18.5 to 58 kg, were used in the study. Goats were determined to be healthy on the basis of history, physical examination findings, total protein concentrations, and Hct values. Food was withheld for 16 hours prior to anesthesia; however, goats were permitted access to water.

Each goat was studied on 4 occasions by use of a randomized crossover design. A minimum washout period of 8 days was permitted between treatments.

Experimental protocol—A blood sample was collected from each goat prior to anesthesia induction to determine plasma magnesium concentration. Anesthesia was induced

<table>
<thead>
<tr>
<th>MAC</th>
<th>Minimum alveolar concentration</th>
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<tr>
<td>NMDA</td>
<td>N-methyl-D-aspartate</td>
</tr>
<tr>
<td>ISOMAC</td>
<td>MAC of isoflurane</td>
</tr>
<tr>
<td>ETISO</td>
<td>End-tidal isoflurane concentration</td>
</tr>
<tr>
<td>MACB</td>
<td>Baseline MAC</td>
</tr>
<tr>
<td>LD</td>
<td>Loading dose</td>
</tr>
<tr>
<td>CRI</td>
<td>Constant rate infusion</td>
</tr>
<tr>
<td>MACT</td>
<td>MAC after treatment</td>
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From the Departments of Large Animal Clinical Sciences (Queiroz-Castro, Redua, Doherty), Small Animal Clinical Sciences (Egger), and Comparative Medicine (Rohrbach, Cox), College of Veterinary Medicine, University of Tennessee, Knoxville, TN 37996-4550. Address correspondence to Dr. Queiroz-Castro.
with isoflurane in oxygen via a face mask attached to a circle anesthetic system. After induction, a cuffed endotracheal tube was inserted and anesthesia was maintained with isoflurane in oxygen (3 L/min) by use of a small animal anesthetic machine.

Goats were placed in left lateral recumbency and ventilated to maintain an end-tidal partial pressure of carbon dioxide of 25 to 30 mm Hg. The ETISO and end-tidal partial pressure of carbon dioxide were monitored continually with an infrared sidestream gas analyzer.

Gas samples were collected from the Y-piece at a flow rate of 50 mL/min. The analyzer was calibrated at the beginning of each experiment by use of commercial gas supplied by the manufacturer (1% isoflurane in 5% CO\textsubscript{2} and 60% N\textsubscript{2}O). Body temperature was measured with an esophageal thermometer, and a circulating water heating blanket was used to maintain body temperature within a range considered normal (38.5° to 39.6°C).

A 20-standard wire gauge catheter was inserted percutaneously into the medial branch of the rostral auricular artery, and blood pressure was monitored continuously by use of a disposable transducer and displayed electronically.

The middle sternum was considered as the zero point when goats were in left lateral recumbency. A 16-standard wire gauge catheter was inserted into a jugular vein, and lactated Ringer’s solution was administered by use of an infusion pump at a rate of 3 mL/kg/h.

Approximately 45 minutes after induction of anesthesia, and with the ETISO held constant at 1.3 vol% for at least 20 minutes, the MAC\textsubscript{o} for isoflurane was determined. A noxious stimulus, which consisted of clamping a hoof between the jaws of vulsellum forceps, was used. The forceps was closed tightly to the first or second ratchet, depending on the hoof size, just below the coronary band, and the hoof was moved continuously for 1 minute or until purposeful movement occurred. Purposeful movement was defined as gross movement of the head or extremities. Coughing, straining, stiffening, or chewing was not considered a purposeful movement. If purposeful movement was detected, the ETISO was increased by 0.1 vol%; otherwise, it was decreased by 0.1 vol%, and the stimulus was reapplied after a 20-minute equilibration period.

The order in which hooves were clamped was randomized. The ISOMAC was defined as the mean of ETISO values at which movement did and did not occur.

The MAC\textsubscript{T} was determined in triplicate, and the mean value was used for statistical analysis.

Following MAC\textsubscript{o} determination, 4 treatments were administered IV as follows: saline (0.9% NaCl) solution (LD, 30 mL/h); CRI, 60 mL/h), magnesium sulfate (LD, 1 mg/kg; CRI, 10 mg/kg/h), ketamine (LD, 1 mg/kg; CRI, 25 mg/kg/min), and magnesium sulfate (LD, 50 mg/kg; CRI, 10 mg/kg/h) combined with ketamine (LD, 1 mg/kg; CRI, 25 mg/kg/min).

Each LD was made up to a final volume of 30 mL in saline solution. The LD of magnesium sulfate was infused over 20 minutes. The LD of ketamine was infused during the last 3 minutes of saline solution or magnesium infusion. All LDs were immediately followed by a CRI consisting of 60 mL in saline solution.

The determination of MAC\textsubscript{T} began 45 minutes after administration of the LD was initiated. The MAC\textsubscript{T} was determined in triplicate, and the mean value was used for statistical analysis.

Drug analysis—Ketamine analysis was performed by use of reversed-phase high-performance liquid chromatography. The system consisted of a separations module and a UV detector. Separation was attained on a 4.6×150-mm (5-μm) column preceded by a 3-μm guard column. The mobile phase was a mixture of 0.03M potassium dihydrogen phosphate buffer (pH, 6) and acetonitrile. The mixture was pumped at a gradient, starting at 89% potassium dihydrogen phosphate, and 11% acetonitrile and was adjusted linearly to 87% potassium dihydrogen phosphate and 13% acetonitrile for over 17 minutes, followed by a return to initial conditions. The flow rate was varied from 1.0 to 1.2 to 1.0 mL/min in conjunction with the mobile phase gradient. Ultraviolet absorbance was measured at 205 nm.

Standard curves for plasma analysis were prepared by spiking untreated goat plasma with ketamine, which produced a linear concentration range of 50 to 7,000 ng/mL. Spiked standards were treated exactly as plasma samples for ketamine determination. Recovery ranged from 83% to 92%. Intra-assay variability ranged from 0.3% to 6.8%, and interassay variability ranged from 7.3% to 10.1%.

Analysis of total magnesium concentrations was performed by use of a commercially available colorimetric assay on the basis of the reaction of magnesium with xylidyl blue in an alkaline solution containing EGTA.

Statistical analysis—A mixed-model ANOVA was used to compare differences among treatment groups for MAC\textsubscript{o}, MAC\textsubscript{T}, and the percentage change in MAC. Percentage change in MAC was calculated as \((\text{MAC}_{\text{T}} – \text{MAC}_{\text{o}})/\text{MAC}_{\text{o}}\) × 100. Independent variables included in the model were treatment, week, treatment×week, and time to measurement of MAC\textsubscript{o} or MAC\textsubscript{T}. Goat was included as a random variable in the model. A comparison of the difference between MAC\textsubscript{T} and MAC\textsubscript{o} among treatment groups was performed by use of the same model, but the time to baseline was substituted for time to MAC\textsubscript{T}. Effect of treatment on ketamine and magnesium concentration in plasma at the time of MAC\textsubscript{T} determination was evaluated with the same ANOVA model that included time to MAC\textsubscript{T}.

In all models, a Tukey-Kramer multiple range test was used to determine statistical significance among various treatments. Results of statistical comparisons to evaluate differences among treatment groups for MAC\textsubscript{o}, MAC\textsubscript{T}, percentage change in MAC, and plasma concentrations of ketamine and magnesium are reported as least squares means ± SEM. Descriptive statistics for time to MAC\textsubscript{o} and time to MAC\textsubscript{T} are reported as mean ± SD. A value of \(P < 0.05\) was considered significant in all tests.

Results

Mean MAC\textsubscript{o} for the 4 treatments was 1.06 ± 0.02 vol%, and MAC\textsubscript{T} did not differ significantly (\(P > 0.05\)) among treatment groups (Table 1). Ketamine significantly decreased ISOMAC by 28.7 ± 3.7%, and administration of ketamine combined with magnesium sulfate significantly decreased ISOMAC by 21.1 ± 4.1%. Treatments with ketamine and ketamine combined with magnesium sulfate were not significantly different from each other. Treatment with saline solution and magnesium sulfate did not significantly change ISOMAC.

Mean time for determination of MAC\textsubscript{o} and MAC\textsubscript{T} was 204 ± 20 minutes and 204 ± 22 minutes, respectively. Time to determine MAC or the order of treatment did not significantly affect MAC\textsubscript{o} or MAC\textsubscript{T}. No significant difference was detected among treatments for time to extubation following the end of...
The mechanism of MAC reduction by ketamine is not clear and could be attributable to its analgesic or sedating actions. Ketamine has sedating effects in humans, as determined by its ability to enhance propofol-induced sedation\(^1\) and cause somnolence in awake humans.\(^2\) The analgesic effects of ketamine are well established. The intraoperative use of a low dose (10 μg/kg/min) of ketamine in dogs, followed by postoperative administration of a CRI (2 μg/kg/min) of ketamine, significantly decreased pain scores without causing obvious sedation.\(^5\) In another study\(^7\) in dogs, ketamine infusion caused a significant increase in the bispectral index, yet, paradoxically, ISOMAC was concurrently decreased.

In the study reported here, magnesium did not significantly change ISOMAC, and this result is not consistent with results of a study\(^7\) in rats. In that study, IV administration of magnesium decreased the MAC of halothane in rats by 20% to 60% in a dose-dependent, nonlinear manner. Doses of magnesium used, however, were high (3.5, 5, and 7 mg/kg/min), and plasma concentrations ranged from 4.86 to 15.76 mg/dL.\(^12\) In the study reported here, the mean plasma magnesium concentrations of goats ranged between 2.4 and 3.6 mg/dL.

Table 2—Plasma concentrations of ketamine and magnesium at the time of MAC determination in 8 goats before treatment (MAC\(_B\)) and after treatment (MAC\(_T\)) with saline (0.9% NaCl) solution (LD, 30 mL/20 min; CRI, 60 mL/h), magnesium sulfate (LD, 50 mg/kg, IV; CRI, 10 mg/kg/h), ketamine (LD, 1 mg/kg, IV; CRI, 25 μg/kg/min), and magnesium sulfate (LD, 50 mg/kg, IV; CRI, 10 mg/kg/h) combined with ketamine (LD, 1 mg/kg, IV; CRI, 25 μg/kg/min).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Ketamine (μg/mL)</th>
<th>Magnesium (mg/dL)</th>
</tr>
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<tbody>
<tr>
<td>Ketamine</td>
<td>0.592 ± 0.02</td>
<td>NA</td>
</tr>
<tr>
<td>Magnesium</td>
<td>2.41 ± 0.04</td>
<td>2.41 ± 0.09</td>
</tr>
<tr>
<td>Ketamine and magnesium</td>
<td>0.573 ± 0.02</td>
<td>2.38 ± 0.09</td>
</tr>
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Concentrations are given as least squares means ± SEM and are based on values from 3 blood samples from each goat at the time of MAC\(_B\) determination. Mean plasma concentrations of ketamine and magnesium did not differ between groups. NA = Not applicable.

Discussion

The baseline MAC of isoflurane determined in the study reported here is consistent with that of previous studies.\(^6,11\) Ketamine decreased ISOMAC by 28.7%, and the mean plasma ketamine concentration at the time of MAC determination was 0.592 μg/mL. Results of a previous study\(^7\) in goats indicate a 50% reduction in ISOMAC at a plasma ketamine concentration of 1.53 μg/mL.

A dose-dependent effect of ketamine on MAC reduction has been reported for isoflurane in dogs and halothane in horses.\(^11\) Comparison of the MAC reduction of isoflurane between goats and dogs indicates that goats may be more sensitive to the MAC-reducing effects of ketamine, as a plasma ketamine concentration of 1.1 μg/mL was necessary to achieve a similar percentage reduction (26%) in dogs.\(^7\) In horses, a plasma ketamine concentration > 1 μg/mL was necessary to decrease the MAC of halothane and the reduction in MAC began to plateau at a plasma concentration of 2 μg/mL. Differences among species in the response to ketamine are possible; alternatively, ketamine may interact differently with isoflurane than with halothane.

In the study reported here, the mean plasma magnesium concentrations of goats ranged between 2.4 and 3.6 mg/dL. Mean plasma concentrations of ketamine were 0.592 and 0.573 μg/mL in goats receiving magnesium sulfate and ketamine combined with magnesium sulfate, respectively. Mean plasma concentrations of magnesium were 2.41 and 2.38 mg/dL in goats receiving magnesium and ketamine, respectively. Mean plasma concentrations of magnesium and ketamine did not differ between groups. Mean plasma concentrations of magnesium did not significantly change ISOMAC, and this result is not consistent with results of a study\(^7\) in rats. In that study, IV administration of magnesium decreased the MAC of halothane in rats by 20% to 60% in a dose-dependent, nonlinear manner. Doses of magnesium used, however, were high (3.5, 5, and 7 mg/kg/min), and plasma concentrations ranged from 4.86 to 15.76 mg/dL. In the study reported here, the mean plasma magnesium concentration at the time of MAC determination was never greater than the magnesium values (2.4 to 3.6 mg/dL) before administration, either following the LD or during the CRI. The lack of increase in magnesium plasma concentration was an unexpected finding. A possible explanation is that the volume of distribution or clearance of magnesium is different in ruminants than in humans. The doses used in the study reported here were based on information obtained from studies\(^11,12\) performed in humans, and pharmacokinetic differences may account for the failure to increase magnesium plasma concentrations above physiologic values.

A potentially confounding factor in the aforementioned study\(^7\) in rats is that the high plasma concentrations of magnesium may have interfered with the estimation of MAC. High plasma magnesium concentrations are known to cause a decrease in acetylcholine release at the neuromuscular junction and a decrease in anesthesia. The mean arterial blood pressure was ≥ 80 mm Hg at all times (range, 80 to 115 mm Hg).

Mean plasma concentrations of ketamine were 0.592 and 0.573 μg/mL in goats receiving ketamine and ketamine combined with magnesium sulfate, respectively. Mean plasma concentrations of magnesium were 2.41 and 2.38 mg/dL in goats receiving magnesium sulfate and ketamine combined with magnesium sulfate, respectively (Table 2). Mean plasma concentrations of ketamine and magnesium did not differ between groups. The plasma magnesium concentration in awake goats ranged between 2.4 and 3.6 mg/dL.
in the responsiveness of the postjunctional membrane to acetylcholine. These effects cause muscle weakness, which could result in a decreased responsiveness to noxious stimulation and, thereby, an overestimation of the reducing effects of magnesium on MAC. Neuromuscular blockade was not monitored in that study, but rats did not have any signs of ataxia after recovering from halothane anesthesia.

An area of controversy in the determination of plasma magnesium concentrations is whether ionized or total magnesium should be measured. Because the ionized fraction is the active form, determination of the ionized concentration would appear to be most appropriate; however, it has been found that IV administration of magnesium sulfate in anesthetized dogs increases the ionized and total plasma magnesium concentrations uniformly. Thus, measurement of total magnesium concentration was determined to be appropriate for our study. It is possible that the concentration of ionized magnesium increased, although the total magnesium concentration did not, but given the results of the aforementioned study, it seems unlikely. The total magnesium concentration should change with administration of magnesium via infusion, and the fact that it did not may reflect the disposition of magnesium sulfate in goats.

Results of studies performed in animals and humans have yielded conflicting information about the anesthetic effects of magnesium. Magnesium was originally thought to have analgesic properties in humans. However, it has since been reported that the effects of magnesium were attributable to hypoxia, hypercapnia, and muscle weakness, and these effects were misinterpreted as resulting from general anesthesia. When respiratory support was maintained in humans, there was no CNS depression, even at high plasma concentrations of magnesium.

Results of a study examining the MAC of sevoflurane in humans indicate that MAC increased after IV administration of magnesium sulfate (30 or 50 mg/kg). In that study, magnesium was administered prior to the induction of anesthesia and patients experienced some unpleasant adverse effects, including excitement, after administration of a magnesium bolus, which increased their stress levels. The authors postulated that increased stress caused the increase in sevoflurane MAC. The plasma magnesium concentration was not reported in that study.

Telci et al reported a significant reduction in the requirements for propofol, remifentanil, and vecuronium by administration of magnesium (LD, 30 mg/kg; CRI, 10 mg/kg/h) during total IV anesthesia in humans. The exact mechanism of magnesium's contribution to anesthesia was not clear in that study.

Differences in the anesthetic drugs used (volatile vs injectable) and species differences in response to magnesium may account for some of the discrepancies among study results. In the study reported here, plasma concentrations of magnesium may not have been high enough to reduce MAC.

Although results of the study reported here indicated that magnesium did not decrease ISOMAC in goats, magnesium appears to have important interactions with volatile anesthetics in the CNS. Thomas et al reported a time-dependent increase in the MAC of halothane (27%) and sevoflurane (22% to 30%) in rats after 12 and 17 days of eating a magnesium-deficient diet. This finding is, perhaps, paradoxical because hypomagnesemia can also cause muscle weakness, which could be interpreted as a reduction of MAC. In that study, neuromuscular blockade was not monitored, but the MAC was increased, suggesting that muscle relaxation did not occur.

Ketamine combined with magnesium decreased ISOMAC by 21.1%, and this reduction in MAC was not significantly (P = 0.17) different from that with ketamine alone (28.7%). Magnesium did not provide an additive effect with ketamine, as had been expected. Magnesium may have prevented ketamine from binding to the NMDA receptors. Results of 2 in vitro pharmacology studies indicate that administration of ketamine and magnesium in combination had a super-additive interaction at the NMDA receptors and enhanced the CNS inhibitory potency of volatile anesthetics. However, results of other in vitro studies with NMDA receptors suggested that the binding sites for ketamine and magnesium may overlap, and such overlap could result in the ability of magnesium to protect the NMDA channel from blockade by ketamine and reduce the effect of ketamine. Results of in vivo studies in rats indicate that hypomagnesemia induced by dietary privation of magnesium was associated with an increased sensitivity to ketamine. Authors of the previous study hypothesized that ketamine could more readily gain access to the binding site on the NMDA receptor in the presence of low plasma magnesium concentrations and that the channel could remain blocked for a prolonged time by ketamine.

In the study reported here, ketamine decreased ISOMAC in goats and verified the clinical impression that it significantly reduces the ETISO needed to maintain surgical anesthesia. Magnesium sulfate, at the doses used in our study, did not reduce ISOMAC in goats or augment the effect of ketamine on MAC.

References

3. Hartman JC, Pagel PS, Proctor LT, et al. Influence of desflur-
rane, isoflurane and halothane on regional tissue perfusion in dogs. 


