

# Effects of subanesthetic doses of ketamine on hemodynamic and immunologic variables in dogs with experimentally induced endotoxemia

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**Objective**—To determine the effects of ketamine hydrochloride on hemodynamic and immunologic alterations associated with experimentally induced endotoxemia in dogs.

**Animals**—9 mixed-breed dogs.

**Procedures**—In a crossover study, dogs were randomly allocated to receive ketamine (0.5 mg/kg, IV, followed by IV infusion at a rate of 0.12 mg/kg/h for 2.5 hours) or control solution (saline [0.9% NaCl] solution, 0.25 mL, IV, followed by IV infusion at a rate of 0.5 mL/h for 2.5 hours). Onset of infusion was time 0. At 30 minutes, lipopolysaccharide (LPS; 1 µg/kg, IV) was administered. Heart rate (HR), systolic arterial blood pressure (SAP), plasma tumor necrosis factor (TNF)-α activity, and a CBC were evaluated.

**Results**—Mean SAP was significantly reduced in dogs administered ketamine or saline solution at 2 and 2.5 hours, compared with values at time 0. However, there was no significant difference between treatments. At 1, 2, and 2.5 hours, dogs administered ketamine had a significantly lower HR than dogs administered saline solution. Although plasma TNF-α activity significantly increased, compared with values at time 0 for both groups, ketamine-treated dogs had significantly lower peak plasma TNF-α activity 1.5 hours after LPS administration. All dogs had significant leukopenia and neutropenia after LPS administration, with no differences detected between ketamine and saline solution treatments.

**Conclusions and Clinical Relevance**—Administration of a subanesthetic dose of ketamine had immunomodulating effects in dogs with experimentally induced endotoxemia (namely, blunting of plasma TNF-α activity). However, it had little effect on hemodynamic stability and no effect on WBC counts. (*Am J Vet Res* 2008;69:228–232)

Gram-negative bacteria are the most common cause of sepsis in dogs.<sup>1–4</sup> During sepsis with gram-negative bacteria, LPS (ie, endotoxin), the glycolipid component of the cell wall of gram-negative bacteria, is released into the bloodstream. Endotoxin then binds to receptors on inflammatory cells, which leads to activation of NF-κB and results in formation of proinflammatory mediators, such as TNF-α.<sup>5</sup> Ultimately, systemic manifestations of endotoxemia (such as fever, tachycardia, and hypotension) develop.

Ketamine is a dissociative anesthetic and competitive antagonist of the NMDA receptor.<sup>6</sup> Ketamine has

## ABBREVIATIONS

LPS	Lipopolysaccharide
NF-κB	Nuclear factor-kappa B
TNF	Tumor necrosis factor
NMDA	N-methyl-D-aspartate
HR	Heart rate
SAP	Systolic arterial blood pressure

several advantageous qualities, compared with qualities of other anesthetic drugs, including unique cardiostimulatory, analgesic, and immunomodulatory effects. Because ketamine has a positive hemodynamic effect,<sup>7</sup> it has been suggested<sup>8,9</sup> as an ideal anesthetic for septic or critically ill dogs that require surgery. Additionally, subanesthetic doses of ketamine provide effective analgesia and have been recommended<sup>10,11</sup> for the management of postoperative pain in dogs.

The immunomodulating effects of ketamine have been reported for several species.<sup>12–16</sup> Ketamine ameliorates NF-κB activation and production of proinflammatory cytokines (such as TNF-α) in mice with experimentally induced endotoxemia.<sup>12,13</sup> Furthermore, ketamine can prevent leukocyte-endothelial cell adhesion in rats,<sup>16</sup> which may in turn decrease neutrophilic

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infiltration of tissues and thus decrease tissue damage. Ketamine also prevents endotoxin-induced hypotension and metabolic acidosis and, most importantly, decreases fatalities in rats.<sup>14,15</sup>

To the authors' knowledge, the potential hemodynamic and immunomodulatory protective effects of ketamine have not been evaluated in dogs. We hypothesized that subanesthetic doses of ketamine would ameliorate the hemodynamic and immunologic perturbations associated with low-grade endotoxemia in dogs. In the study reported here, healthy dogs were administered ketamine and a control solution prior to administration of low-dose endotoxin. Hemodynamic differences between the ketamine and control treatments were evaluated via changes in HR and SAP, whereas immunologic differences were evaluated by means of WBC counts and plasma TNF- $\alpha$  activity.

## Materials and Methods

**Animals**—Nine mixed-breed sexually intact purpose-bred dogs that were 8 months to 5 years old (mean, 1.7 years) and weighed 19.1 to 26 kg (mean, 22.8 kg) were used in the study. There were 3 males and 6 females. All dogs were considered to be in good health on the basis of results of physical examination and screening laboratory tests, including a CBC, serum biochemical analysis, and urinalysis. Dogs were housed in a standard manner in animal facilities accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International, and all care was provided in accordance with the principles outlined by the National Institutes of Health.<sup>17</sup> All experimental procedures were reviewed and approved by the Animal Care and Use Committee of the University of Missouri, Columbia.

**Experimental procedures**—A randomized, crossover study was conducted. Dogs were randomly assigned to initially receive ketamine ( $n = 5$  dogs) or a control solution (4) on the first day of the study. Food was withheld from dogs prior to each day of the study on which infusions were administered.

A 20-gauge catheter<sup>a</sup> was placed in a cephalic vein of each dog 1 hour before initiation of the study. Ketamine hydrochloride<sup>b</sup> (0.5 mg/kg, IV as a bolus, followed by an IV infusion at a rate of 0.12 mg/kg/h [diluted with saline {0.9% NaCl} solution to achieve a rate of 0.5 mL/h] for 2.5 hours) or saline solution (0.25 mL/kg, IV as a bolus, followed by an IV infusion at a rate of 0.5 mL/h for 2.5 hours) was administered to the appropriate treatment group. Thirty minutes after onset of the infusion, LPS from *Escherichia coli* 0127:B8<sup>c</sup> (1  $\mu$ g/kg) was administered IV as a bolus.<sup>18</sup> Dogs that became hemodynamically unstable (SAP < 60 mm Hg or HR < 50 or > 200 beats/min) were given an IV bolus of saline solution (15 mL/kg), which was repeated as needed to maintain hemodynamic stability. Heart rate and SAP were measured at 0 (onset of infusion), 0.5, 1, 1.5, 2, 2.5, and 4.5 hours after onset of infusion. Blood samples were collected at 0, 1, 1.5, 2, 2.5, and 4.5 hours after onset of control or ketamine infusions. After the 4.5-hour study period was completed, the dogs were monitored until all vital clinical indices had returned to

their respective reference ranges without the need for fluid support. After a 10-day washout period, the treatments were reversed and the study repeated.

**Hemodynamic evaluation**—Indirect Doppler<sup>d</sup> ultrasonographic SAP was evaluated by use of a technique described elsewhere.<sup>19</sup> A minimum of 4 measurements was obtained at each time point; the mean for these measurements was calculated to provide the SAP used for analysis. Heart rate was measured by palpation of femoral pulse or cardiac auscultation.

**Immunologic evaluation**—Blood samples were collected via jugular venipuncture into EDTA and lithium heparin collection tubes for use in CBC and TNF- $\alpha$  activity analysis, respectively. An automated counter<sup>e</sup> was used to determine the CBC. Additionally, a differential cell count of 300 cells was performed on blood films stained by use of Wright's stain; counts were conducted by an investigator who was unaware of the source of each sample.

Blood samples collected for measurement of TNF- $\alpha$  activity were immediately placed on ice and centrifuged (300  $\times$  g for 6 minutes at 15°C). The plasma was then removed, immediately frozen, and stored at -80°C until analysis. Plasma TNF- $\alpha$  activity was measured by use of a modification of a technique described elsewhere.<sup>20,21</sup> Briefly, cells from a mouse fibroblast cell line (L929) were cultured on 96-well plates. Diluted plasma samples were added to the wells in triplicate. After incubation for 20 hours in minimal essential medium with 1% horse serum and actinomycin D<sup>c</sup> (3  $\mu$ g/mL), cell numbers were assessed via a 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (ie, MTT) colorimetric assay. Absorption was measured at a wavelength of 570 nm. Mouse TNF- $\alpha$ <sup>f</sup> was used to construct a standard curve that allowed quantification of TNF- $\alpha$  activity in the dog samples.

**Statistical analysis**—Statistical analysis was accomplished by use of commercially available software.<sup>g</sup> A mixed-linear, repeated-measures model with a compound symmetry covariance structure was fitted to the data; variance variables were estimated by use of the restricted maximum likelihood estimation of the covariance method. Numeric data for each of the treatments were analyzed by use of the Fisher least significant difference test. Categorical data were evaluated by use of a Fisher exact test. A value of  $P < 0.05$  was considered significant.

## Results

**Animals**—All dogs remained conscious and ambulatory during ketamine infusion. All dogs developed signs of endotoxemia (mild lethargy and mild vomiting or diarrhea) that lasted for 4 or 5 hours after LPS administration. By 6 hours after LPS administration, attitude, activity, and food and water intake returned to normal in all dogs, and all vital clinical indices were within the expected reference ranges. No long-term sequelae were detected in any of the dogs.

**Hemodynamic variables**—Significant differences were not detected in SAP or HR between the treatments at time 0, nor did administration of ketamine or saline

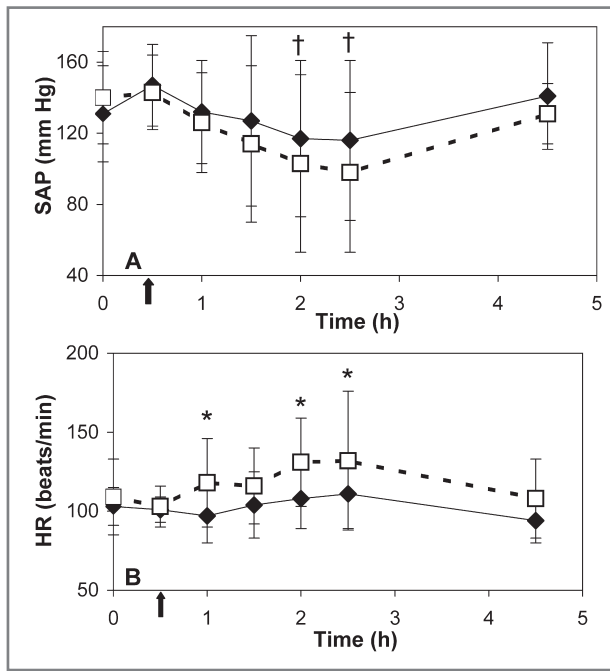


Figure 1—Mean  $\pm$  SD SAP (A) and HR (B) for 9 dogs administered an IV infusion of ketamine hydrochloride (black diamonds) and saline (0.9% NaCl) solution (white squares) followed by an injection of LPS. Ketamine and saline solution administration began at time 0, and LPS was administered 30 minutes later (arrow). \*Within a time point, value differs significantly ( $P < 0.05$ ) between ketamine and saline solution treatments. †Within a time point, values for each treatment differ significantly ( $P < 0.05$ ) from the value for each treatment at time 0.

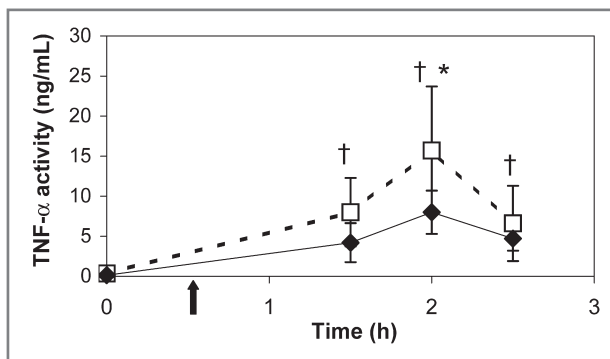


Figure 2—Mean  $\pm$  SD plasma TNF- $\alpha$  activity for 9 dogs administered an IV infusion of ketamine (black diamonds) and saline solution (white squares) followed by injection of LPS. See Figure 1 for remainder of key.

solution alter the HR or SAP prior to the onset of LPS administration at 0.5 hours (Figure 1). Mean SAP was significantly ( $P = 0.03$ ) reduced in the ketamine and saline solution treatments at 2 and 2.5 hours, compared with SAP at time 0. However, there was no significant difference between ketamine and saline treatments. The number of dogs that required IV administration of fluid because of severe hypotension (SAP  $< 60$  mm Hg) did not differ significantly between treatments (control solution, 3/9; ketamine, 1/9). At 1, 2, and 2.5 hours, dogs administered ketamine had a significantly ( $P = 0.04$ ) lower HR, compared with the HR for dogs administered saline solution.

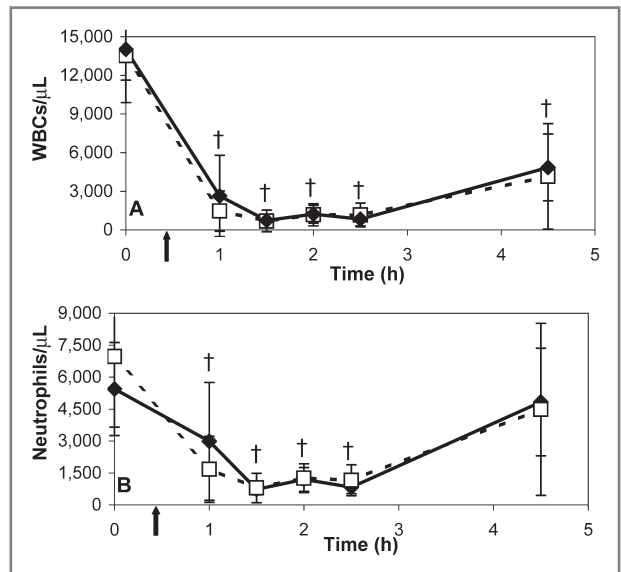


Figure 3—Mean  $\pm$  SD WBC count (A) and neutrophil count (B) for 9 dogs administered an IV infusion of ketamine (black diamonds) and saline solution (white squares) followed by an injection of LPS. See Figure 1 for remainder of key.

**Immunologic variables**—Plasma TNF- $\alpha$  activity was significantly ( $P = 0.005$ ) increased at all times after LPS administration in all dogs for both treatments. Mean TNF- $\alpha$  plasma activity peaked at 2.0 hours (1.5 hours after LPS administration) for both treatments (Figure 2). Although TNF- $\alpha$  activity increased (compared with values at time 0) after saline solution or ketamine administration, dogs administered ketamine had significantly ( $P < 0.001$ ) lower peak plasma TNF- $\alpha$  activity at 2.0 hours, compared with the value for dogs when administered saline solution.

White blood cell counts did not differ significantly between the treatments at time 0. All dogs receiving ketamine or saline solution were significantly ( $P < 0.001$ ) leukopenic and neutropenic after LPS administration, compared with WBC counts at time 0 (Figure 3). The nadir for the total leukocyte count and neutrophil count was detected at 1.5 hours. We did not detect significant differences between values for dogs when administered ketamine or saline solution.

## Discussion

Lipopolysaccharide stimulates macrophages to produce TNF- $\alpha$  by activating intracellular signaling mechanisms (eg, NF- $\kappa$ B).<sup>22</sup> Similar to results for other studies,<sup>18,23-25</sup> dogs in the study reported here had increases in TNF- $\alpha$  activity after LPS administration. Continuous IV infusion of subanesthetic doses of ketamine successfully blunted LPS-induced plasma TNF- $\alpha$  activity. This finding is consistent with findings in rats that subanesthetic doses of ketamine have an inhibitory effect on NF- $\kappa$ B activation and TNF- $\alpha$  production.<sup>13,14</sup> This finding is especially interesting in light of the fact that higher plasma TNF- $\alpha$  activity has been associated with a poorer prognosis in dogs with naturally developing sepsis.<sup>26</sup> Attenuating the production of TNF- $\alpha$  with

ketamine may hold promise as a novel anti-inflammatory strategy for dogs with endotoxemia.

Studies of rodents *in vivo*<sup>27</sup> and of human neutrophils *ex vivo*<sup>16</sup> have provided evidence of the attenuating effects of ketamine on endotoxin-induced integrin expression and venular leukocyte adherence; however, ketamine administration did not alter leukopenia or neutropenia resulting from LPS administration in our dogs. It is possible that the inhibitory effects of ketamine on leukocyte adherence may be short-lived and therefore may have been missed because of the time of sample collections in the study. Alternatively, our ketamine dose may have been insufficient to alter integrin expression and subsequent leukocyte adherence in dogs. Nevertheless, it appears improbable that a clinically important and sustained change in leukocyte numbers is associated with subanesthetic doses of ketamine.

Ketamine has been recommended for anesthesia in canine patients with sepsis because of its cardiostimulatory properties.<sup>7,8,14,15,28,29</sup> Similar to results in other studies,<sup>24,30</sup> SAP decreased after LPS administration in the dogs reported here, but there was no difference between the ketamine and saline solution treatments. Heart rate was higher in dogs when administered saline solution, compared with HR when dogs were administered ketamine. We speculate that a higher HR may have been necessary to maintain blood flow and blood pressure, assuming that systemic vascular resistance was less when dogs were infused with saline solution. Regardless, the subanesthetic dose of ketamine minimally affected measured cardiovascular variables. It is possible that other doses of LPS or ketamine may have elicited different effects. This is a reasonable possibility because the cardiostimulatory effects of ketamine appear to be a dose-dependent event.<sup>7</sup>

The dose of ketamine used in the study reported here was selected on the basis that it was the dose commonly used for pain management in dogs.<sup>11</sup> Because many animals that develop gram-negative bacterial infections do so in association with conditions that cause pain (eg, septic abdomen, trauma, or surgery),<sup>31,32</sup> immunomodulation from an analgesic drug is a particularly appealing effect and may affect morbidity or mortality rates in dogs with naturally developing sepsis. We determined that ketamine at a subanesthetic dose had immunomodulating effects in dogs after low-dose endotoxin administration (namely, blunting of plasma TNF- $\alpha$  activity), but it had little effect on hemodynamic stability and no effect on WBC counts. Because TNF- $\alpha$  is believed to be an important mediator of many of the clinically detrimental consequences of endotoxemia, additional study of ketamine infused at subanesthetic doses in dogs after the onset of endotoxemia and in dogs with naturally developing endotoxemia is warranted.

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- b. Ketaset, Fort Dodge Animal Health, Fort Dodge, Iowa.
- c. Sigma-Aldrich, St Louis, Mo.
- d. Parks Medical Electronics, Las Vegas, Nev.
- e. Coulter Electronics, Hialeah, Fla.
- f. Fischer Scientific International, Pittsburgh, Pa.
- g. SAS, version 9, SAS Institute Inc, Cary, NC.

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