

# Ketamine Inhibits Endotoxin-induced Shock in Rats

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**Background:** Cytokines and nitric oxide are believed to participate importantly in the pathogenesis of endotoxin-induced shock. Several investigators have documented that ketamine attenuates production of cytokines and nitric oxide in endotoxemia, but little is known concerning hemodynamic effects of the drug in this state. The objective of the current study was to assess the potential modifying effects of ketamine in endotoxemia.

**Methods:** The authors randomly assigned 40 rats to one of four equal groups: endotoxin alone, receiving *Escherichia coli* endotoxin (15 mg/kg, administered intravenously); saline control, receiving saline only; ketamine alone, receiving ketamine (10 mg · kg<sup>-1</sup> · h<sup>-1</sup>, administered intravenously); pretreatment, with ketamine administration initiated before the endotoxin exposure; and posttreatment, with ketamine initiated 2 h after endotoxin. During the 5 h after endotoxin injection, hemodynamics, acid-base status, and plasma concentrations of tumor necrosis factor  $\alpha$  and interleukin 6 were assessed in each group.

**Results:** Endotoxin injection produced progressive hypotension, metabolic acidosis, and a large increase in the plasma cytokine concentrations. This hemodynamic and cytokine responses to endotoxin were completely abolished in the pretreatment group and modestly suppressed in the posttreatment group. In the absence of endotoxin, ketamine did not modify these responses.

**Conclusion:** Ketamine administration inhibited hypotension, metabolic acidosis, and cytokine responses in rats injected with endotoxin. The results suggest that judicious use of ketamine as an anesthetic agent may offer advantages in endotoxemia.

ENDOTOXIN shock, a common problem in patients with endotoxemia that often resists even intensive medical treatment, is characterized by profound hypotension, progressive metabolic acidosis, and dysfunction of multiple organs. Although its pathophysiology is not well defined, cytokines and nitric oxide (NO) are considered important in mediating the cardiovascular disturbance.<sup>1-3</sup> Circulating endotoxin increases production and release of tumor necrosis factor  $\alpha$  (TNF- $\alpha$ ) and interleukin 6 (IL-6), which are dilators of resistance vessels in skeletal muscle.<sup>4</sup> When macrophages are activated by endotoxin or cytokines, the inducible NO synthase gene

is expressed. In turn, NO synthase is produced and, once activated, continuously releases large amounts of NO, which has a vasodilating effect.<sup>5,6</sup> Enhanced production of NO contributes importantly to the cardiovascular dysfunction.

Several investigators have documented that ketamine attenuates production and release of cytokines in endotoxemia *in vitro*.<sup>7-9</sup> In addition, ketamine has been reported to inhibit accumulation of nitrite, a metabolite of NO, in a dose-dependent fashion in endotoxin-activated macrophages,<sup>10</sup> and also to inhibit induction of NO synthase activity and protein expression by endotoxin.<sup>11</sup> We therefore hypothesized that ketamine could inhibit development of cardiovascular dysfunction by attenuation of cytokine responses in sepsis. To test this hypothesis, we studied the effects of ketamine on hemodynamics, acid-base status, and plasma concentrations of TNF- $\alpha$  and IL-6 in endotoxin-exposed rats.

## Materials and Methods

### Animal Preparation

All experimental procedures were approved by the Animal Care Committee of Kanazawa University School of Medicine and were in accordance with the National Institutes of Health guidelines for animal use. Male Wistar rats (n = 40), weighing 373 ± 14 g (mean ± SD), were anesthetized with an intraperitoneal injection of pentobarbital sodium (30 mg/kg). Ventilation was performed through a tracheotomy. The femoral artery was cannulated to monitor blood pressure and to draw blood samples. Lactated Ringer's solution containing a muscle relaxant (pancuronium bromide, 0.02 mg/ml) and pentobarbital sodium (0.5 mg/ml) was infused continuously at a rate of 10 ml · kg<sup>-1</sup> · h<sup>-1</sup> through a femoral vein cannula, because animals anesthetized with this dose of pentobarbital alone did not demonstrate evidence of escape behavior. The heart rate was recorded from lead II of the electrocardiogram. The rats were connected to a pressure-controlled ventilator (Servo 900B, Siemens-Elema, Solna, Sweden) delivering 100% oxygen at a frequency of 30 breaths/min with an inspiratory:expiratory ratio of 1:1. The animals then were rested for at least 15 min to stabilize hemodynamics, followed by baseline readings of heart rate and systolic arterial pressure (SAP). In addition, data on arterial blood gases and acid-base status were obtained (ABL-520, Radiometer, Copenhagen, Denmark).

### Experimental Protocols

After baseline measurements, animals were allocated randomly to one of five groups, three of which received endotoxin (n = 8 per group).

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**Endotoxin Group.** Endotoxin shock was induced by a bolus injection of endotoxin (15 mg/kg). We used lipopolysaccharide prepared from *Escherichia coli* (O111:B4; Difco Laboratories, Detroit, MI) as endotoxin.

**Saline Control Group.** Animals received a bolus injection of 0.9% saline (1.0 mg/kg) followed by an infusion of 0.9% saline. Animals in this group were not exposed to endotoxin.

**Ketamine Group.** Animals received a bolus injection of 0.9% saline (1.0 ml/kg), while ketamine was administered intravenously ( $10 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ ). Animals in this group were not exposed to endotoxin and served as controls to assess the sympathomimetic effects of ketamine.

**Pretreatment Group.** This group received an infusion of ketamine ( $10 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ ). After initiation of infusion, a bolus dose of endotoxin (15 mg/kg) was given.

**Posttreatment Group.** This group received a bolus dose of endotoxin (15 mg/kg) and, beginning 2 h later, received an infusion of ketamine ( $10 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ ). Rectal temperature was maintained between 36 and 38°C using a heating pad. An arterial blood sample (0.25 ml) was drawn hourly throughout the 5-h observation period for the measurement of arterial pH, oxygen tension, carbon dioxide tension, and bicarbonate concentration. Furthermore, arterial blood samples (1.5 ml) were drawn for the measurement of plasma cytokine concentrations at 2, 4, and 5 h after the endotoxin or saline injection. The total amount of blood drawn from each animal was 5.5 ml over 5 h.

#### Sample Analysis

Blood used for determinations of cytokine concentrations was centrifuged for 10 min at 3,000g at 4°C. Plasma was decanted and stored at -70°C until analysis. Cytokine concentrations (TNF- $\alpha$  and IL-6) were measured using enzyme-linked immunosorbent assays (BioSource, Camarillo, CA). The lower limits of detection for TNF- $\alpha$  and IL-6 were 4.5 and 7.0 pg/ml, respectively.

#### Statistical Analysis

Data are presented as mean  $\pm$  SD. Differences between groups at baseline were analyzed by Student unpaired *t* test and Mann-Whitney U test. Hemodynamic and cytokine changes during the study were analyzed using two-way analysis of variance with repeated measures followed by *post hoc* test (Bonferroni method). Statistical significance was defined at  $P < 0.05$ . Statistical analyses were performed using the StatView application (version 5.0, Macintosh; Abacus Concepts, Berkeley, CA).

## Results

#### Hemodynamics

No significant differences were noted in baseline heart rate or SAP between groups (fig. 1). Endotoxin injection

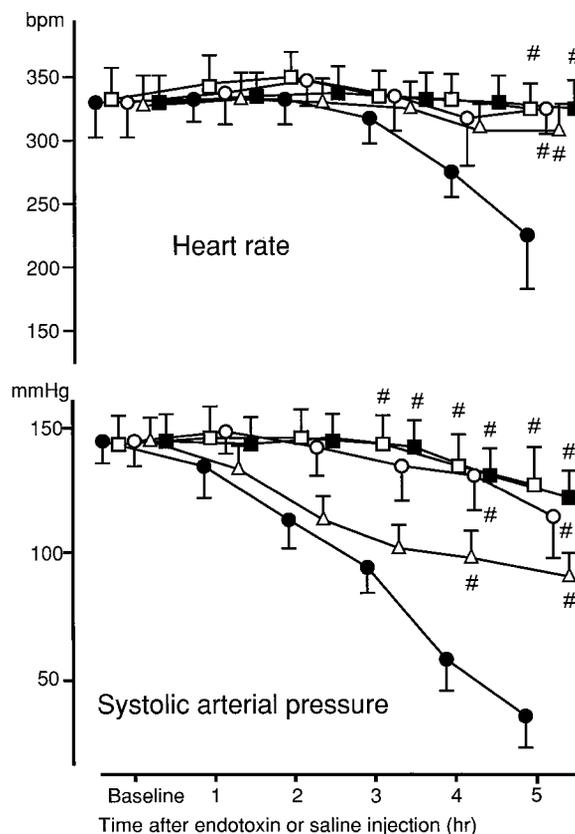


Fig. 1. The heart rate (*top*) and systolic arterial pressure (*bottom*) at baseline and after injection of endotoxin or saline (mean  $\pm$  SD). Closed circles, endotoxin group; closed squares, saline control group; open squares, ketamine group; open circles, pretreatment group; open triangle, posttreatment group. # $P < 0.05$  versus endotoxin group.

decreased SAP in the endotoxin-alone and posttreatment groups but not in the saline control, ketamine-alone, or pretreatment groups. Five hours after endotoxin injection, SAP decreased by 77, 11, 43, 9, and 18% in the endotoxin-alone, saline control, posttreatment, ketamine-alone, and pretreatment groups, respectively. No significant differences in SAP occurred between the latter two groups at any time point during the experimental period. Thus, the hemodynamic response differed among the three groups receiving endotoxin. In contrast, the response to ketamine did not differ between the latter two groups, which received the same dose of the agent.

#### Blood Gases

Carbon dioxide and oxygen tensions did not differ significantly between the five groups at any point during the experimental period (table 1). The endotoxin injection decreased arterial pH and the bicarbonate concentration in the endotoxin-alone and posttreatment groups. At 5 h after injection, a significantly lower arterial pH was observed in the endotoxin-alone group than in the other four groups. Thus, acid-base balance was maintained better in groups receiving ketamine.

**Table 1. Arterial Blood Gas Values at Baseline and after the Endotoxin Injection**

	Baseline	Time after Injection (h)				
		1	2	3	4	5
<b>pHa</b>						
Endotoxin group	7.47 ± 0.07	7.46 ± 0.08	7.36 ± 0.10	7.25 ± 0.05	7.20 ± 0.09	7.11 ± 0.12
Saline control group	7.51 ± 0.06	7.52 ± 0.11	7.48 ± 0.12	7.46 ± 0.13	7.47 ± 0.10	7.45 ± 0.10*
Ketamine group	7.54 ± 0.10	7.59 ± 0.08	7.53 ± 0.11	7.47 ± 0.15	7.47 ± 0.07	7.46 ± 0.08*
Ketamine pretreatment group	7.50 ± 0.07	7.53 ± 0.12	7.50 ± 0.11	7.45 ± 0.13	7.41 ± 0.13	7.38 ± 0.11*
Ketamine post-treatment group	7.54 ± 0.10	7.46 ± 0.11	7.37 ± 0.10	7.33 ± 0.12	7.32 ± 0.12	7.30 ± 0.14*
<b>Pao<sub>2</sub> (mmHg)</b>						
Endotoxin group	481 ± 65	517 ± 46	503 ± 75	508 ± 75	518 ± 60	496 ± 6
Saline control group	500 ± 49	488 ± 43	493 ± 45	511 ± 60	490 ± 75	475 ± 71
Ketamine group	479 ± 75	500 ± 60	507 ± 60	522 ± 55	506 ± 55	519 ± 52
Ketamine pretreatment group	483 ± 35	491 ± 59	478 ± 66	462 ± 51	459 ± 70	494 ± 70
Ketamine post-treatment group	488 ± 70	488 ± 43	493 ± 45	511 ± 60	490 ± 65	486 ± 70
<b>Paco<sub>2</sub> (mmHg)</b>						
Endotoxin group	30 ± 8	28 ± 10	30 ± 12	30 ± 8	26 ± 8	25 ± 11
Saline control group	30 ± 6	28 ± 9	28 ± 10	30 ± 10	28 ± 8	28 ± 10
Ketamine group	24 ± 7	21 ± 6	21 ± 9	24 ± 7	25 ± 6	24 ± 9
Ketamine pretreatment group	26 ± 7	28 ± 12	28 ± 9	29 ± 8	28 ± 9	27 ± 9
Ketamine posttreatment group	27 ± 4	24 ± 10	24 ± 7	29 ± 10	28 ± 10	28 ± 10

All data are mean ± SD. n = 8/group.

\* P < 0.05 versus endotoxin group.

pHa = arterial pH; Pao<sub>2</sub> = arterial oxygen pressure; Paco<sub>2</sub> = arterial carbon dioxide pressure.

### Plasma Cytokine Concentrations

All baseline values were similar in the five groups (fig. 2). Endotoxin injection increased the TNF- $\alpha$  concentration in the endotoxin-alone, pretreatment, and posttreatment groups, but the concentration remained significantly less in the pretreatment group than in the other two groups at 2 h after injection. Plasma concentrations of IL-6 became elevated in the endotoxin-alone and posttreatment groups. Significantly lower concentrations resulted in the pretreatment and posttreatment groups than in the endotoxin-alone group.

### Discussion

Injection of endotoxin alone in the present experiments produced progressive hypotension, metabolic acidosis, and a large increase in plasma cytokine concentrations. Ketamine modified the hemodynamic and cytokine responses; when ketamine administration was initiated immediately before endotoxin injection, the responses were completely abolished; when ketamine was initiated 2 h after the endotoxin injection, the responses were modestly suppressed. Ketamine administration alone had minimal effects on hemodynamics and cytokine responses. Thus, ketamine administration inhibited development of hypotension and increase of cytokine concentrations in endotoxin-exposed rats, representing the most important findings of the current study.

Previous reports<sup>4,12,13</sup> also have described cardiovascular dysfunction after endotoxin injection, including hypotension and metabolic acidosis. Endotoxin has both

direct and indirect cardiovascular effects, directly depressing myocardial contractility while indirectly producing peripheral vasodilation. This direct negative inotropic effect has been demonstrated in isolated heart muscle preparations.<sup>14,15</sup> The indirect effect on arterial smooth muscle results from increased production of cytokines and NO.<sup>1-3</sup> Circulating endotoxin increases production and release of TNF- $\alpha$  and IL-6, which are vasodilating agents.<sup>4</sup> In addition, endotoxin and the two cytokines activate macrophages, resulting in induction of NO synthase and release of large amounts of NO.<sup>5,6</sup> Increased NO production and release have been implicated in cardiovascular dysfunction and diminished response to vasoconstrictors in endotoxemia.<sup>16</sup>

Of particular interest is the inhibitory effect of ketamine on the production or release of IL-6 *in vivo*. In our endotoxin-alone group, pronounced increases in plasma concentrations of TNF- $\alpha$  and IL-6 were observed. In the pretreatment group, the cytokine response was abolished, and these concentrations remained significantly lower than in the endotoxin-alone group. Ketamine has been reported to have a suppressive effect on the TNF- $\alpha$  production after endotoxin exposure *in vivo*,<sup>17</sup> and the significantly lower concentrations of IL-6 demonstrated here may simply be a consequence of suppressed TNF- $\alpha$  production. In our posttreatment group, a marked increase in plasma TNF- $\alpha$  concentration was seen, as also occurred in the endotoxin-alone group; however, a significantly lower concentration of IL-6 was present 5 h after endotoxin injection (*i.e.*, 3 h after initiation of ketamine administration). The difference between the endotoxin-alone and posttreatment groups cannot be

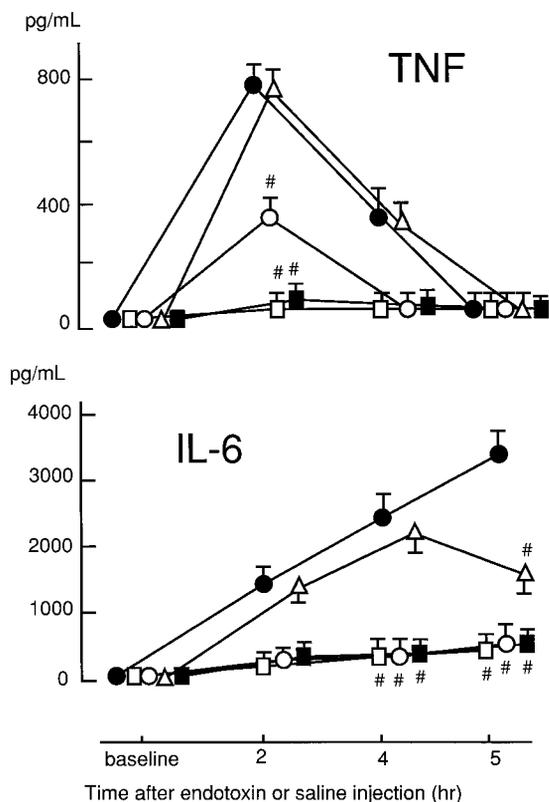


Fig. 2. Changes of plasma tumor necrosis factor  $\alpha$  (TNF; top) and interleukin 6 (IL-6; bottom) at baseline and after injection of endotoxin or saline (mean  $\pm$  SD). Closed circles, endotoxin group; closed squares, saline control group; open squares, ketamine group; open circles, pretreatment group; open triangle, posttreatment group. # $P < 0.05$  versus endotoxin group.

explained simply by the suppressive effect of ketamine on TNF- $\alpha$  production. Kawasaki *et al.*<sup>9</sup> reported that ketamine inhibited not only endotoxin-induced IL-6 but also TNF- $\alpha$ -induced IL-6 production *in vitro*. The current study indicated that ketamine independently affects production or release of IL-6 in endotoxemia *in vivo*.

Our findings that ketamine pretreatment attenuated the cytokine response suggest that prevention of an increase in TNF- $\alpha$  and IL-6 was important in averting development of cardiovascular dysfunction. However, even in the posttreatment group, in which increases in these cytokines were similar to those in the endotoxin-alone group, SAP was preserved, and better acid-base balance was maintained. Therefore, inhibition of cardiovascular dysfunction in our pretreatment and posttreatment groups cannot be explained solely by prevention of increases in TNF- $\alpha$  and IL-6. Differences in NO production or other factors may be more important. Further studies need to focus on this point.

Ketamine has been recommended for induction of anesthesia and sedation in patients with circulatory failure because of its actions in increasing sympathetic nervous system activity, which tends to maintain blood pressure and preserve cardiovascular function.<sup>18</sup> This sympathomimetic property may have influenced our re-

sults somewhat, potentially obscuring the precise mechanisms responsible for inhibition of endotoxin shock in the current study. However, in the ketamine-alone group, administration of the agent produced no remarkable changes in hemodynamic parameters. Furthermore, other investigators have reported that, in patients with shock, including endotoxin shock, induction of anesthesia with ketamine can cause marked cardiovascular depression.<sup>19</sup> These considerations led us to conclude that preservation of hemodynamics observed in the pretreatment and posttreatment groups resulted from antiinflammatory effects rather than from sympathomimetic properties of the agent.

In the current study, we injected the high dose of endotoxin, which was approximately 2  $\times$  the 50% lethal dose.<sup>20</sup> Our previous study showed that this dose of endotoxin caused hypotension within 4 h after endotoxin injection in rats.<sup>21</sup> We used this dose to evaluate the cytokine responses in endotoxin-induced shock. Moreover, another important question is whether a dose-response relation exists between ketamine and hemodynamics and cytokine responses. The administration dose of ketamine (10 mg  $\cdot$  kg<sup>-1</sup>  $\cdot$  h<sup>-1</sup>) was relative high compared with those required to produced anesthesia in humans. However, the required maintenance dose of ketamine is known to be species-dependent and may be as high as 12 mg/kg in dogs.<sup>22</sup> Thus, the dose of ketamine in the current study is not surprising.

In summary, the current study showed that administration of ketamine inhibited hypotension, metabolic acidosis, and cytokine responses in rats receiving a single intravenous bolus dose of endotoxin. An inhibitory effect was found even when initiation of treatment was delayed until 2 h after endotoxin exposure. Although the mechanisms responsible for the inhibitory effects require further investigation, our results suggest that ketamine as an anesthetic agent may offer advantages in endotoxemia.

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