

## ***Hemodynamic and Metabolic Manifestations of Acute Endotoxin Infusion in Pigs with and without the Malignant Hyperthermia Mutation***

Shailesh Kumar V. Musley, Ph.D.,\* David S. Beebe, M.D.,† Vijaya Komanduri, M.S.,‡ Paul A. Iaizzo, Ph.D.,§ Kumar G. Belani, M.D.||

**Background:** The hypermetabolic state induced by acute endotoxemia and malignant hyperthermia (MH) may be indistinguishable. The aims of this study were (1) to investigate the differences between MH and sepsis, (2) to determine whether acute endotoxemia can trigger MH, and (3) to establish the effects of dantrolene in these two disorders.

**Methods:** Three groups of swine were studied. All pigs were invasively monitored and initially anesthetized with nontriggering agents. A placebo MH-susceptible group (n = 5) received normal saline whereas the endotoxin groups (MH-susceptible, n = 6; MH-negative, n = 4) received intravenous endotoxin (250 µg/kg total) during 2.5 h. Halothane (1.5%) and succinylcholine (2-4 mg/kg) were then administered, followed by two doses of dantrolene (4 mg/kg total).

**Results:** Endotoxin infusion resulted in pulmonary hypertension and systemic hypotension in pigs with and without the MH mutation, but did not trigger MH. Halothane and succinylcholine triggered MH, evidenced by a markedly higher oxygen consumption in the MH-susceptible pigs that received endotoxin (325 ± 196 ml/min) and those that did not (374 ± 110 ml/min) compared to

the MH-negative pigs (69 ± 15 ml/min,  $P < 0.0009$ ), as well as muscular rigidity in the susceptible animals. Dantrolene reversed these changes. Three of the six MH-susceptible pigs that received endotoxin died; two died soon after triggering and one after dantrolene administration. In contrast, none of the MH-negative pigs or the MH-susceptible pigs that did not receive endotoxin died (0 of 9 vs. 3 of 6,  $P = 0.044$ ).

**Conclusion:** Endotoxemia does not trigger MH, but may worsen outcome if it occurs. (Key words: Calcium; dantrolene; fever; sepsis.)

THE clinical presentation of malignant hyperthermia (MH) and septicemia may be indistinguishable. Both can present with hyperthermia, tachycardia, tachypnea, and acidosis.<sup>1</sup> MH is an inherited disorder that phenotypically is described as a subclinical myopathy that progresses to a severe hypermetabolic crisis on exposure to potent anesthetic agents, succinylcholine, exercise, or severe stress. The underlying myopathy is caused by functional alterations in the regulation of the sarcoplasmic reticulum that causes an acute increase in the intracellular calcium concentration ( $\text{Ca}^{2+}$ ). In swine, MH susceptibility results from a single amino acid substitution (Arg to Cys) at position 615 of the sarcoplasmic reticulum ryanodine receptor ( $\text{Ca}^{2+}$  release channel). The cause is more heterogeneous in humans.<sup>2</sup> In both species, dantrolene can reverse an MH episode.<sup>2,3</sup> Dantrolene acts to reduce or stabilize the intracellular free calcium.<sup>4</sup> To be effective, dantrolene must be given early, while muscle blood flow is still present. It may have to be administered before a diagnosis can be accurately established, and could be administered to patients in whom sepsis is the cause of hyperpyrexia rather than MH.<sup>1</sup>

The aims of this study were (1) to study the differences in the metabolic, hemodynamic, and biochemical manifestations during acute endotoxemia in normal pigs and those susceptible to MH, (2) to determine whether the stress of acute endotoxemia can trigger MH in susceptible swine, and (3) to determine whether dantrolene administration has any adverse effects in the presence of endotoxemia.

\* Postdoctoral Scholar, Department of Anesthesiology.

† Associate Professor, Department of Anesthesiology.

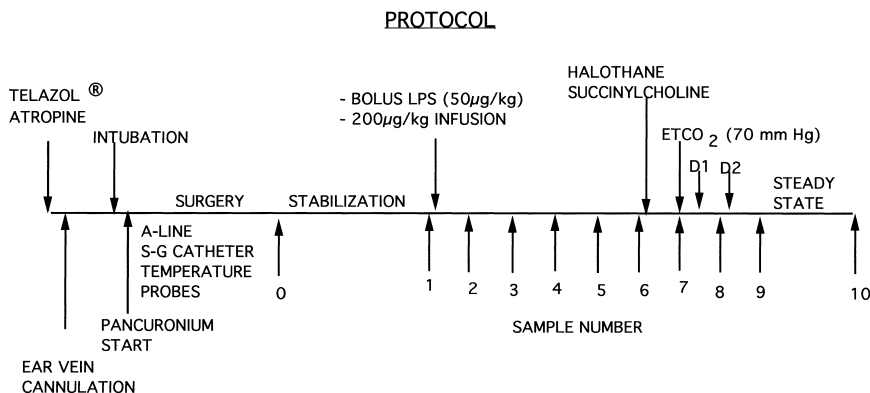
‡Scientist, Department of Anesthesiology.

§Associate Professor, Departments of Anesthesiology and Physiology, the Biomedical Engineering Institute.

|| Professor, Departments of Anesthesiology and Pediatrics; and Clinical Professor, Department of Anesthesiology, University of California, San Francisco, California.

Received from the Departments of Anesthesiology; and Physiology, the Biomedical Engineering Institute; and Pediatrics, the University of Minnesota Medical School, Minneapolis, Minnesota. Submitted for publication November 30, 1998. Accepted for publication May 5, 1999. Supported by the Department of Anesthesiology research funds, University of Minnesota Medical School, Minneapolis, Minnesota. Presented in part at the national meeting of the Association of University Anesthesiologists, Washington, DC, April 17-20, 1997, and at the annual meeting of the American Society of Anesthesiologists, San Diego, CA, October 18-22, 1997.

Address reprint requests to Dr. Belani: Professor, Departments of Anesthesiology and Pediatrics, Box 294, B515 Mayo Memorial Building, 420 Delaware Street Southeast, Minneapolis, Minnesota 55455. Address electronic mail to: belan001@tc.umn.edu



**Fig. 1.** A schematic diagram of the experimental protocol used in this study.

## Methods

All animal studies were approved by the University of Minnesota Animal Care and Use Committee and conformed to the published guidelines of the National Institutes of Health on the use of laboratory animals. These experiments were performed on normal swine and purebred pietrane swine with the MH genotype (University of Minnesota Rosemount Agricultural Experiment Station, West Rosemount, Minnesota). The genotype was confirmed by linkage analyses. On the day of the study, general anesthesia was induced with an intramuscular injection of Telazol (tiletamine HCL and zolazepam HCL, 12 mg/kg; Fort Dodge Animal Health, Fort Dodge, IA) and atropine 0.1 mg/kg. An intravenous line was established in an ear vein, and anesthesia was maintained with continuous infusion of Telazol and nitrous oxide (70%). Pancuronium was used for skeletal muscle relaxation. The animals (body weight  $\approx 24$  kg) were intubated and mechanically ventilated (partial pressure of carbon dioxide [ $P_{aCO_2}$ ] between 35–45 mmHg, ventilator model 90013, Ohmeda, Madison, WI). Respiratory gases were monitored with a Nellcor N-2500 unit (Nellcor, Pleasanton, CA). A balloon-tipped pulmonary artery catheter (model 93-132-5F; Edwards Swan-Ganz Thermodilution Catheter, Irvine, CA) was inserted in the right external jugular vein and advanced into the pulmonary artery. This catheter was used for measurements of pulmonary artery pressure, blood temperature, collection of mixed venous blood, and the determination of cardiac output by the thermodilution method (cardiac output computer 9520A; Edwards Laboratories, Santa Ana, CA). An average of three cardiac outputs was obtained during each data sampling period. An indwelling femoral arterial line was placed for blood pressure monitoring and measurement of arterial blood gases.

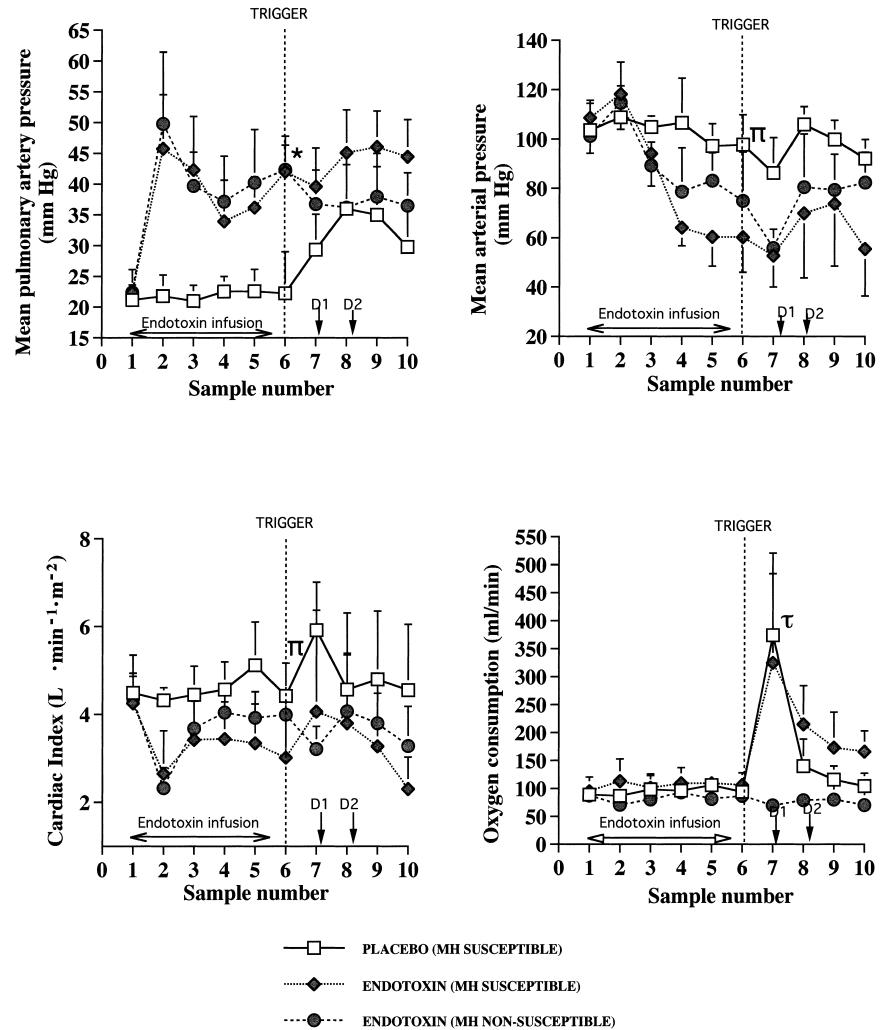
After surgery, the animals were allowed a 30-min sta-

bilization period, during which time they were maintained normothermic ( $\approx 37 \pm 0.5^\circ\text{C}$ ) by using a forced-air cover positioned over the torso and lower limbs, which was connected to a forced-air warming device (model 500; Bair Hugger, Augustine Medical Inc., Eden Prairie, MN) set to deliver air at approximately  $40^\circ\text{C}$ . The arterial and venous blood pressure of oxygen ( $P_{O_2}$ ), pressure of carbon dioxide ( $P_{CO_2}$ ), pH, and electrolytes were determined from fresh blood samples using an IL 1400 BGElectrolytes Analyzer (Instrumentation Laboratory, Lexington, MA), with temperature compensation. Samples of arterial and venous blood were also tested for lactate levels by the University of Minnesota Hospital laboratories (Minneapolis, MN). To determine the cardiac index, the body surface area was calculated using the Dubois equation:  $BSA = BW^{0.425} \cdot \text{length}^{0.725} \cdot 0.007184$ . The ambient temperature was approximately  $23^\circ\text{C}$ .

After preparation and stabilization, the placebo MH-susceptible group ( $n = 5$ ), received equivalent volumes of normal saline, whereas the endotoxin groups (MH-susceptible,  $n = 6$ ; MH-negative,  $n = 4$ ) received *Escherichia coli* endotoxin dissolved in normal saline (LPS; 0111::B4, Difco, Detroit, MI), in an intravenous bolus dose of 50  $\mu\text{g}/\text{kg}$  followed by 200  $\mu\text{g}/\text{kg}$  intravenously infused over 2.5 h (see Protocol, fig. 1). After endotoxin or placebo infusion, the pigs were challenged with triggering agents (halothane 1.5% + succinylcholine 2–4 mg/kg). Five to 10 min after exposure to triggering agents, or when the end-tidal carbon dioxide ( $ET_{CO_2}$ ) reached 70 mmHg or more, 2 mg/kg dantrolene was administered in two doses (D1 and D2). At the same time dantrolene was given, halothane administration was discontinued. All measurements were repeated every 30 min until triggering and every 15 min after triggering for 1 h. After data acquisition, surviving animals were killed.

## ENDOTOXEMIA AND MALIGNANT HYPERTHERMIA

Fig. 2. Mean pulmonary artery pressure, mean arterial pressure, cardiac index, and oxygen consumption values for the three groups of animals. There were originally six pigs in the endotoxin (malignant hyperthermia [MH]-susceptible) group, four in the endotoxin (MH-nonsusceptible) group, and five in the placebo (MH-susceptible) group. Two pigs in the endotoxin (MH-susceptible) group died soon after triggering, and one died after receiving the second dose of dantrolene (after sample 9). \* $P < 0.0035$  for changes from baseline (sample 1) in the endotoxin (MH-susceptible) and endotoxin (MH-nonsusceptible) pigs versus the placebo (MH-susceptible) pigs.  $\pi P < 0.009$  for changes from baseline (Sample 1) in the endotoxin (MH-susceptible) group versus the placebo (MH-susceptible) group.  $\tau P < 0.0009$  for changes from the point before triggering (sample 6) in the both the endotoxin (MH-susceptible) and the placebo (MH-susceptible) pigs versus the endotoxin (MH-nonsusceptible) pigs.



### Statistical Analysis

Data were reported as the mean  $\pm$  SD. The change from the baseline values after endotoxin administration for each parameter were compared among the three groups immediately before administering triggering agents (sample 6) using analysis of variance. To determine the effects of triggering, the changes from these values (sample 6) caused by the triggering agents, but before the administration of dantrolene (sample 7), were compared among the groups using analysis of covariance (ANCOVA). The changes from the post-triggering values (sample 7) after dantrolene administration (sample 9) were also compared among the groups using analysis of covariance. Log transformation of values was performed if the transformed data met the equal variances criteria better than the original data. If the F test for the model was significant, pairwise comparisons were evaluated for significant (least squares)

mean differences among each group. Adjustments for multiple comparisons were made using the Bonferroni method. Therefore, significance was defined as  $P < 0.0167$ . To evaluate the overall mortality difference among the three groups, the Fischer exact test was used.

### Results

In MH-susceptible and nonsusceptible animals, endotoxin infusion resulted in decreases in the mean arterial pressure cardiac index, with a marked increase in the pulmonary artery pressure (fig. 2). Both the mixed venous and the  $ET_{CO_2}$  tensions increased in the groups that received endotoxin, with a decrease in pH (fig. 3). However, the blood temperatures were not different among the groups (fig. 4). There was no evidence that intravenous endotoxin triggered MH.

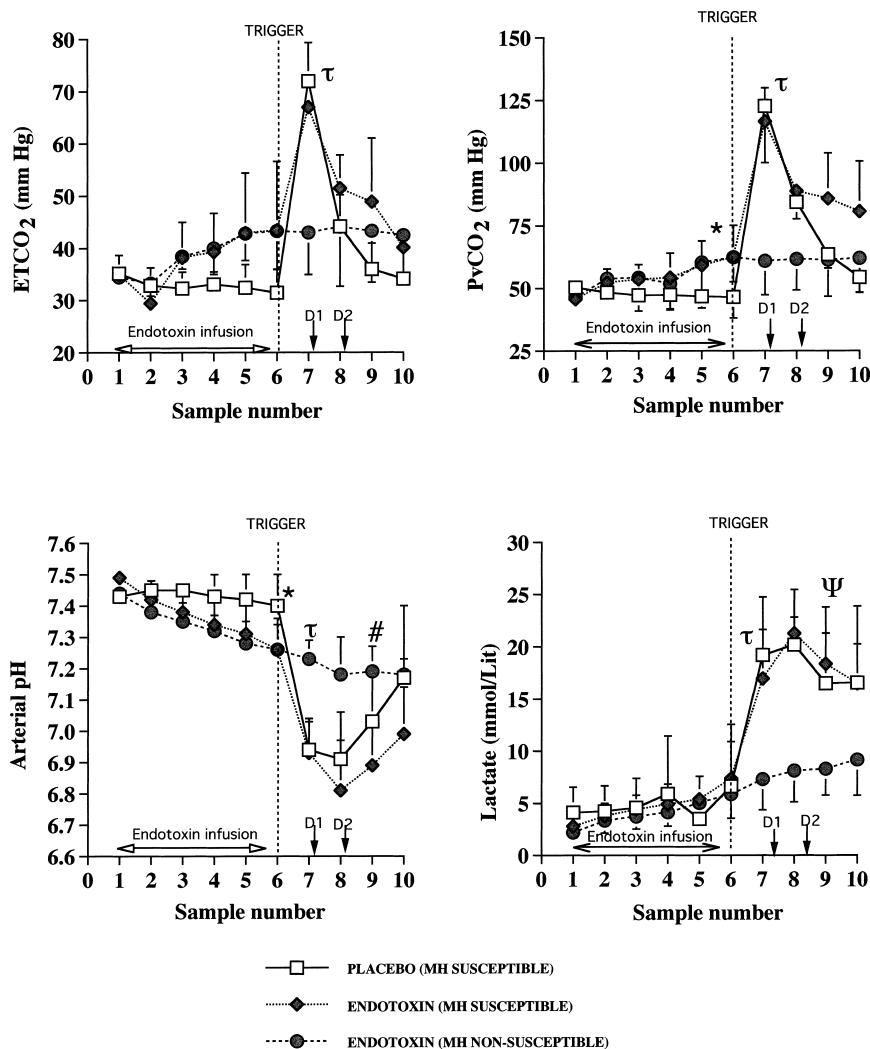


Fig. 3. End-tidal carbon dioxide tension ( $ET_{CO_2}$ ), pulmonary venous carbon dioxide tension ( $Pv_{CO_2}$ ), arterial pH, and serum lactate values for the three groups of animals. \* $P < 0.0096$  for changes from baseline (sample 1) for endotoxin (malignant hyperthermia [MH]-susceptible) pigs versus placebo (MH-susceptible) pigs.  $\tau P < 0.0376$  (not significant) for endotoxin (MH-nonsusceptible) pigs versus placebo (MH-susceptible) pigs.  $\tau P < 0.0011$  for changes from the point before triggering (sample 6) for both the placebo (MH-susceptible) pigs and the endotoxin (MH-susceptible) pigs versus endotoxin (MH-nonsusceptible) animals. # $P < 0.0124$  for changes from the point after triggering (sample 7) after dantrolene administration (sample 9) for endotoxin (MH-susceptible) versus endotoxin (MH-nonsusceptible) pigs.  $\psi P < 0.0067$  for changes from the point after triggering (sample 7) after dantrolene administration (sample 9) for the endotoxin (MH-susceptible) pigs versus the endotoxin (MH-nonsusceptible) group of pigs.  $\psi P < 0.017$  (not significant) for the endotoxin (MH-nonsusceptible) pigs versus the placebo (MH-susceptible) pigs.

Administration of the triggering agents halothane and succinylcholine caused marked increases in the oxygen consumption, mixed venous carbon dioxide levels,  $ET_{CO_2}$ , and serum lactate level in the MH-susceptible pigs, regardless of whether they received endotoxin previously (figs. 2 and 3). The blood temperature increased in the MH-susceptible groups as well, but not as dramatically (fig. 4). Dantrolene began to reverse these changes in both MH-susceptible groups (fig. 3).

All MH-susceptible pigs that did not receive endotoxin survived triggering of MH after discontinuation of halothane and administration of dantrolene. None of the MH-negative swine triggered MH. They also survived after all the triggering agents were discontinued and dantrolene was administered. In contrast, one half of the MH-susceptible pigs who received endotoxin died (3 of 6 vs. 0 of 9;

$P = 0.044$ ). Soon after triggering agents were begun, two of the six MH-susceptible pigs that received endotoxin died from severe hypotension before they could be treated with dantrolene. A third pig in the MH-susceptible group also died from severe hypotension despite the administration of dantrolene.

## Discussion

A hypermetabolic response during anesthesia may spontaneously occur in a number of diverse medical conditions, such as thyrotoxicosis, acute sepsis, neuroleptic malignant syndrome, pheochromocytoma, and MH.<sup>1,5,6</sup>

Malignant hyperthermia is an unexpected event that requires prompt action by the anesthesia care team. During

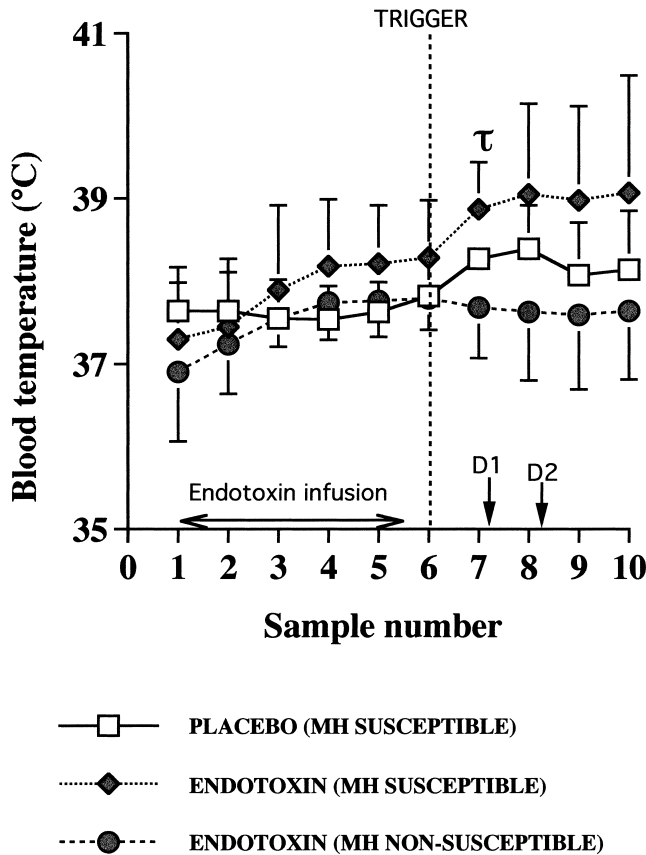


Fig. 4. Blood temperature for the three groups of animals.  $\tau P < 0.005$  for changes from the point before triggering (sample 6) for the endotoxin (malignant hyperthermia [MH]-susceptible) pigs versus the endotoxin (MH-nonsusceptible) animals.

an episode of MH, skeletal muscles increase their oxygen consumption and lactate production, resulting in respiratory and metabolic acidosis, muscle rigidity, and generalized hyperthermia. The final common pathway for the development of MH appears to be an uncontrolled increase in intracellular  $Ca^{2+}$  in skeletal muscle.<sup>2,4</sup>

Dantrolene acts by inhibiting excitation-contraction coupling in the skeletal muscle without affecting neuromuscular transmission or the electrical properties of the muscle. Excitation-contraction coupling results in release of  $Ca^{2+}$  from the sarcoplasmic reticulum. Dantrolene thus diminishes  $Ca^{2+}$  release without affecting re-uptake.<sup>5,7</sup> Dantrolene rapidly halts the increase in metabolism from MH and secondarily results in a return to normal levels of catecholamines and potassium.<sup>8</sup>

In MH-susceptible pigs, there is some evidence that dantrolene acts by affecting the abnormal ryanodine receptor. It may act directly on the ryanodine receptor to diminish  $Ca^{2+}$  release, or at a site adjacent to the

receptor, which affects its function.<sup>9,10</sup> Dantrolene may also interact with the dihydropyridine receptor. This receptor is involved with electrical transmission from the transverse tubules to the ryanodine receptor.<sup>11</sup> Alternatively, dantrolene may act at a receptor or site that counteracts the elevated intracellular  $Ca^{2+}$  without binding to or involving the ryanodine receptor directly.<sup>12</sup> Dantrolene may affect the stability of the membrane of the sarcoplasmic reticulum to counteract the destabilizing effects of halothane as well. Membrane instability may cause a change in the function of the  $Ca^{2+}$  release channel and result in increased intracellular calcium.<sup>13</sup>

Recent studies also suggest that disturbances in  $Ca^{2+}$  regulation are responsible for or contribute to many of the metabolic manifestations of sepsis or endotoxemia, or both. A sustained increase in intracellular  $Ca^{2+}$  in sepsis or endotoxemia may be partly responsible for induction and continuation of multiple organ dysfunction.<sup>14</sup> Song *et al.*<sup>15</sup> demonstrated that intracellular  $Ca^{2+}$  is increased in the aortic smooth muscle in rats administered endotoxin. Endotoxin has been shown to increase intracellular inositol triphosphate levels.<sup>16</sup> Inositol triphosphate may act on a receptor in the sarcoplasmic reticulum similar to the ryanodine receptor that triggers release of  $Ca^{2+}$  into the cytoplasm.<sup>17</sup> However, in our experiment, there was no evidence that endotoxin administration alone triggered MH.

Song *et al.*<sup>15</sup> also suggested that the intracellular  $Ca^{2+}$  be intentionally lowered in septic patients by using intravenous dantrolene. They demonstrated that intravenous dantrolene lowers intracellular  $Ca^{2+}$  in rats that were administered endotoxin. Dantrolene also has reversed some of the metabolic hallmarks of sepsis, such as muscle protein breakdown and plasma lactate formation, in rats made septic by cecal ligation. Dantrolene increased survival in mice administered endotoxin as well.<sup>18</sup> Another study showed that dantrolene did not worsen survival in rats made endotoxemic and was not detrimental to the hemodynamics of dogs given endotoxin.<sup>19</sup> Conversely, dantrolene administration was shown to worsen survival in a model of intraabdominal sepsis in rats.<sup>20</sup>

In humans, dantrolene has been administered without apparent harm to patients in whom sepsis is the cause of hyperpyrexia rather than MH.<sup>1</sup> Dantrolene treatment also was not detrimental to the normal pigs given endotoxin in our experiment, and reversed the MH episode in the susceptible pigs that were not given endotoxin.

However, half of the MH-susceptible pigs that were made septic with endotoxin died from profound hypotension during administration of triggering agents. One pig died in

spite of receiving intravenous dantrolene, although it did begin to reverse the MH episode in the three remaining septic pigs. These results are similar to a report of four patients diagnosed postoperatively as MH susceptible by positive results of halothane contracture tests of either themselves or one of their parents. MH developed in each while undergoing surgery for a septic appendix. Three of the four patients died intraoperatively, and two of the patients died in spite of receiving intravenous dantrolene.<sup>1</sup> This suggests that, although endotoxin itself may not trigger MH, the presence of sepsis worsens mortality.

Perhaps the rise in intracellular  $\text{Ca}^{2+}$  from triggering MH in addition to the increase in  $\text{Ca}^{2+}$  from endotoxemia may be too high for survival in patients or animals that are septic and MH susceptible. Profound hypotension may also occur when halothane and succinylcholine both are used to trigger MH in susceptible animals. This may be caused by the presence of up-regulated extra-junctional nicotinic acetylcholine receptors in MH-susceptible animals. Interaction of succinylcholine with these receptors during triggering can cause systemic hypotension.<sup>21</sup> Because the septic pigs were already hypotensive from endotoxin, they may not have been able to survive an additional diminution in systemic blood pressure resulting from the triggering agents.

In summary, the hemodynamic, metabolic, and biochemical manifestations of acute endotoxin infusion in the presence of nontriggering anesthetics were similar in pigs with and without the MH mutation. Dantrolene treatment resulted in no detrimental effects in the normal swine that received the endotoxin. Despite severe alterations in hemodynamic and metabolic parameters, the stress of endotoxemia did not result in the initiation of an episode of MH in susceptible pigs. However, the presence of endotoxemia increased mortality after triggering in MH-susceptible swine.

The authors thank Mallinckrodt Anesthesia Products Incorporated (St. Louis, Missouri), for providing the temperature probes, and Nellcor Incorporated (Pleasanton, CA) for the use of their multifunction gas analyzer.

## References

1. Strecker G, Adnet P, Forget AP, Krivosic-Horber R: Hyperthermia maligne et état infectieux d'origine appendiculaire. Peut-on les différencier en cours d'intervention? *Ann Fr Anesth Reanim* 1997; 16:234-8
2. Denborough M: Malignant hyperthermia. *Lancet* 1998; 352: 1131-6
3. Harrison GG: Control of the malignant hyperpyrexia syndrome in MHS swine by dantrolene sodium. *Br J Anaesth* 1975; 47:62-5
4. Lopez JR, Allen P, Alamo L, Ryan JF, Jones DE, Sreter F: Dantrolene prevents the malignant hyperthermia syndrome by reducing free intracellular calcium concentration in skeletal muscle of susceptible swine. *Cell Calcium* 1987; 8:385-96
5. Greenberg C: Diagnosis and treatment of hyperthermia in the postanesthesia care unit. *Anesth Clin North Am* 1990; 8:377-97
6. Hunter WAH, Randalls B, Grande CM: Intraoperative hyperthermia. Etiology and treatment. *Prob Anesth* 1994; 8:122-36
7. Lopez JR, Allen PD, Almo L, Jones DE, Sreter FA: Myoplasmic free  $[\text{Ca}^{2+}]$  during malignant hyperthermia episodes in swine. *Muscle Nerve* 1988; 11:82-8
8. Kolb ME, Horne ML, Martz R: Dantrolene in human malignant hyperthermia: A multicenter study. *ANESTHESIOLOGY* 1982; 56:254-62
9. Parness J, Palnitkar SS: Identification of dantrolene binding sites in porcine skeletal muscle sarcoplasmic reticulum. *J Biol Chem* 1995; 270:18465-72
10. Fruen BR, Mickelson JR, Louis CF: Dantrolene inhibition of sarcoplasmic reticulum  $\text{Ca}^{2+}$  release by direct and specific action at skeletal muscle ryanodine receptors. *J Biol Chem* 1997; 272:26965-71
11. El-Hayek R, Parness J, Valdivia HH, Coronado R, Hogan K: Dantrolene and azumolene inhibit  $[\text{H}^3]\text{PN200-110}$  binding to porcine skeletal muscle dihydropyridine receptors. *Biochem Biophys Res Comm* 1992; 187:894-900
12. Palnitkar SS, Mickelson JR, Louis CF, Parness J: Pharmacological distinction between dantrolene and ryanodine binding sites: Evidence from normal and malignant hyperthermia-susceptible porcine skeletal muscle. *Biochem J* 1997; 326:847-52
13. Gaillard S, Rock E, Vignon X, Dufourc EJ, Renou JP:  $^{31}\text{P}$  NMR and freeze fracture studies of sarcoplasmic reticulum membranes from normal and malignant hyperthermic pigs: Effect of halothane and dantrolene. *Arch Biochem Biophys* 1992; 294:154-9
14. Hotchkiss RS, Karl IE: Calcium: A regulator of the inflammatory response in endotoxemia and sepsis. *New Horiz* 1996; 4:58-71
15. Song SK, Karl IE, Ackerman JH, Hotchkiss RS: Increased intracellular  $\text{Ca}^{2+}$ : A critical link in the pathophysiology of sepsis? *Proc Natl Acad Sci U S A* 1993; 90:3933-7
16. Shinji H, Akagawa KS, Tsuji M, Maeda M, Yamada R, Matura K, Yamamoto S, Yoshida T: Liposaccharide-induced biphasic inositol 1,4,5-triphosphate response and tyrosine phosphorylation of 140-kilodalton protein in mouse peritoneal macrophages. *J Immunol* 1997; 158:1370-6
17. Tonner PH, Scholz J, Richter A, Löscher W, Steinfath M, Wappler F, Wlaz P, Hadji B, Roewer N, Schulte Am Esch J: Alterations of inositol polyphosphates in skeletal muscle during porcine malignant hyperthermia. *Br J Anaesth* 1995; 75:467-71
18. Hotchkiss RS, Karl IE: Dantrolene ameliorates the metabolic hallmarks of sepsis in rats and improves survival in a mouse model of endotoxemia. *Proc Natl Acad Sci U S A* 1994; 91:3039-43
19. Beebe DS, Belani KG, Tuohy SE, Sweeney MF, Gillingham K, Komanduri V, Palahniuk RJ: Is dantrolene safe to administer in sepsis? The effect of dantrolene after endotoxin administration in dogs and rats. *Anesth Analg* 1991; 73:289-94
20. Cameron EM, Zhuang J, Menconi MJ, Phipps R, Fink MP: Dantrolene, an inhibitor of intracellular calcium release, fails to increase survival in a rat model of intra-abdominal sepsis. *Crit Care Med* 1996; 24:1537-42
21. Sigg DC, Iaizzo PA: Insights into succinylcholine-induced hypotension and malignant hyperthermia in susceptible swine. *Anesth Analg* 1999; 88 (suppl 2):S383