

Laboratory Report

Porcine Malignant Hyperthermia:

Effect of Dantrolene Sodium on In-vitro Halothane-induced Contraction of Susceptible Muscle

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Halothane-induced contractures in isolated muscle fibers from swine susceptible to malignant hyperthermia (MHS) were significantly less when fibers were incubated in KRB plus 6.2×10^{-6} M dantrolene sodium prior to the administration of 4 per cent halothane. Administration of dantrolene sodium at the time of maximum contraction to MHS fibers in which contractures had been induced by halothane significantly increased the rate of relaxation of these fibers compared with similar fibers not treated with dantrolene sodium. This study indicates possible prophylactic and therapeutic value of dantrolene sodium in malignant hyperthermia and suggests that the previously reported effectiveness of dantrolene sodium in preventing and treating halothane-induced contractures may be due, at least in part, to its direct effect on muscles. (Key words: Hyperthermia, malignant; Neuromuscular relaxants, dantrolene.)

MUSCULAR RIGIDITY is observed in nearly every case of untreated porcine malignant hyperthermia¹ and in approximately two thirds of human cases.² Although the primary defect in malignant hyperthermia is presumed to occur within the muscle cell,^{2,3} an intact local spinal reflex system has recently been demonstrated to be necessary for complete development of the porcine malignant hyperthermia syndrome.⁴

The hypermetabolic state, expressed by rigidity, ATP depletion and heat production, that occurs in malignant hyperthermia-sus-

ceptible (MHS) muscle following exposure to specific triggering anesthetic agents is assumed to be related to persistent elevated myoplasmic calcium concentration.^{2,5} Various mechanisms responsible for this have been proposed, including increased calcium influx,¹ increased permeability of sarcoplasmic reticulum (SR) membrane to calcium,³ decreased rate of uptake of calcium by SR^2 and decreased calcium uptake by mitochondria.⁷

The relatively new muscle relaxant, dantrolene sodium,[†] produces its effect distal to the myoneural junction, and available evidence suggests that this drug interferes with calcium release from the sarcoplasmic reticulum.⁸ A drug directly affecting calcium movement into and out of subcellular structures such as the terminal cisternae of the SR would appear to have a role in the prophylaxis and therapy of malignant hyperthermia. *In-vitro* efficacy of dantrolene sodium in the treatment and prevention of porcine malignant hyperthermia has recently been demonstrated.⁹

In this study, the effect of dantrolene sodium *in vitro* on halothane-induced contractures in muscle from MHS swine has been tested.

Methods and Materials

Malignant hyperthermia-susceptible Poland China swine were identified by halothane and halothane-succinylcholine exposure as previously described.¹ The control

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Received from the College of Veterinary Medicine, Oklahoma State University, Stillwater, Oklahoma. Accepted for publication August 18, 1975. Supported in part by USPHS General Research Support Grant RR-05567 and Ayerst Laboratories, New York, New York.

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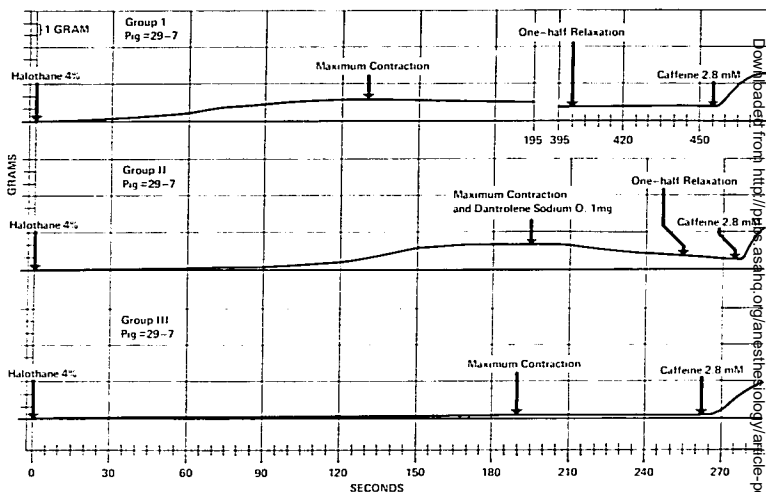


FIG. 1. The effect of dantrolene sodium on halothane-induced contractures in isolated porcine malignant hyperthermia-susceptible muscle. The tracings show changes in tension in grams (Y axis) with time in seconds (X axis). Fibers in Groups I and II were equilibrated in KRB at 1 g tension prior to halothane treatment. Group III fibers were equilibrated in KRB plus 6.2×10^{-6} M dantrolene sodium prior to halothane treatment.

animal was a confirmed negative from the same family as the MHS swine.

Seven or eight muscle biopsy specimens were obtained from each of the five MHS swine used in this study and five muscle biopsy specimens were obtained from the control pig. Isolated muscle fibers were prepared and kept at 37 C in Krebs-Ringer's solution buffered with sodium bicarbonate to pH 7.40 (KRB) through which carbogen (95 per cent O_2 , 5 per cent CO_2) was continuously bubbled. Fibers were tested for contracture response according to the method described by Nelson *et al.*¹

Muscle fibers from each MHS pig were randomly assigned to three treatment groups. Group I fibers ($n = 11$), after an initial 4-5-minute equilibration period in KRB at 1 g tension, were exposed to 4 per cent halothane in carbogen, which was bubbled through the bathing fluid. The response was measured as change in tension, and the interval from the commencement of halothane

treatment to maximum contraction was recorded. The interval from the time of maximum contraction until the fiber preparation tension decreased to a value midway between the initial resting tension and peak tension (one-half relaxation time, fig. 1) was also recorded. To test for muscle contractility, caffeine was then added to the muscle bath to make a final concentration of 2.8 mM.

Group II fibers ($n = 22$) were equilibrated in the muscle bath at 1 g tension and exposed to halothane as were the fibers in Group I. At the time of peak tensile response, dantrolene sodium, 0.1 mg, in alkaline mannitol was added to the muscle bath to provide a final concentration of 6.2×10^{-6} M. Times for the intervals, onset of halothane to peak contraction, and peak contraction to half relaxation were recorded as in Group I. Finally, caffeine, 2.8 mM, was added to the muscle bath to test for muscle contractility.

Group III fibers ($n = 11$) were placed in the muscle bath containing KRB plus 6.2

$\times 10^{-6}$ M dantrolene sodium. After equilibration, as in Groups I and II, 4 per cent halothane vapor was bubbled through the bathing medium. The changes in tension and time to maximum contraction were recorded, and finally, caffeine, 2.8 mM, was added to the preparation.

Control fibers were incubated in KRB, equilibrated, exposed to 4 per cent halothane, then 0.1 mg dantrolene sodium, and finally, 2.8 mM caffeine.

Differences among means were measured using Student's *t* test, with correction, where necessary, for equal variances.

Results

Muscle fibers in Group I had increased tension when exposed to halothane (fig. 1). These increases ranged from 0.53 to 3.00 g, with a mean of 1.60 g (table 1). The maximum contraction occurred, on the average, 145 seconds (range 120–185 seconds) after commencement of halothane administration, and the half relaxation time averaged 296 seconds (range 155–530 seconds).

Group II muscle fibers also had increased tension when exposed to halothane (fig. 1). These responses did not differ significantly from the responses of Group I fibers (table 1); they averaged plus 1.84 g (range 0.75–4.22 g), and the maximum contraction occurred, on the average, 136 seconds after exposure to halothane (range 70–200 seconds). These times were not significantly different from the corresponding times in Group I.

Treatment of the contracted fibers in Group II with dantrolene sodium significantly reduced the half relaxation time (mean 94 seconds, range 53–156 seconds) compared with untreated fibers in Group I ($P < 0.001$).

Fibers in Group III that were equilibrated in KRB plus dantrolene sodium had significantly reduced halothane-induced contractures compared with fibers in both Group I and Group II (fig. 1, table 1). The responses of Group III fibers to halothane averaged 0.33 g (range 0.13–0.67 g), and the maximum contractions occurred later (mean 167 seconds, range 95–223 seconds) than those observed in Groups I and II, although a significant difference ($P < 0.05$) was demonstrated between Group III and Group II only.

All MHS fibers in Groups I, II, and III contracted in the presence of 2.8 mM caffeine (fig. 1). Fibers in Group III apparently had the greatest sensitivity to this test for muscle contractility.

The control fibers failed to contract in the presence of 4 per cent halothane. They also had no response to dantrolene sodium, and caffeine-induced contractures were apparently less than those observed in the MHS fibers.

Discussion

This study indicates that at low concentrations dantrolene sodium confers significant protection against halothane-induced contractions in isolated MHS muscle fibers. It also demonstrates the apparent effectiveness of dantrolene sodium in reversing existing halothane-induced contractures in isolated MHS muscle fibers. This evidence supports the report by Harrison⁹ of the apparent effectiveness of this drug in reversing and preventing the development of halothane-induced malignant hyperthermia in known susceptible swine.

This present study demonstrates that dantrolene sodium prevents or at least attenuates and reverses the functional responses occurring in halothane-induced malignant hyperthermia. There is no direct evidence from this study to indicate that the metabolic changes present in malignant hyperthermia are prevented or reversed by dantrolene sodium.

This study confirms the previously reported hypersensitivity of MHS muscle to halothane-caffeine-induced contractures.^{1,2} Although caffeine was used in this study only to test for muscle viability and contractility, it is interesting that MHS muscle fibers in Groups I and II were apparently less sensitive to caffeine than were fibers in Group III. These differences could be explained by the decreased contractility of muscle fibers in which contractures had already been induced by halothane.

Caffeine induces contraction in normal muscle either by decreasing the rate of calcium re-entry into the SR or by facilitating calcium release from the SR.¹⁰ Both mechanisms would increase intracellular calcium to about

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TABLE 1. Effects of Dantrolene Sodium on Halothane-induced Contractions in Isolated Porcine Malignant Hyperthermia Muscle

	Maximum* Tension Change ΔG (g)	Time, Halothane to Maximum Contraction (Sec)	Time, Contraction One-half Relaxation (Sec)
Group I, halothane			
\bar{X}	1.60†	145.18	296.09§
SE	0.20	6.09	39.96
N	11		
Group II, halothane- dantrolene sodium			
\bar{X}	1.84†	136.00†	94.32§
SE	0.18	6.56	6.84
N	22		
Group III, dantrolene sodium prior to halothane			
\bar{X}	0.33†	167.00†	—
SE	0.05	11.97	—
N	11		

* ΔG = maximum tension-initial tension after exposure to 4 per cent halothane.

† Group III vs. Groups I and II, $P < 0.001$.

‡ Group II vs. Group III, $P < 0.05$.

§ Group I vs. Group II, $P < 0.001$.

the concentration necessary to release troponin-myosin inhibition and initiate the sequence of events that culminates in contraction.

Halothane and other malignant hyperthermia-triggering agents also appear capable of increasing intracellular calcium ion concentration, possibly through effects similar to those of caffeine on the SR and mitochondrial or sarcolemmal membranes.

Dantrolene sodium has been reported both to affect¹¹ and not to affect^{12,13} caffeine-induced contracture responses in isolated amphibian muscle preparations. As suggested by Ellis and Carpenter,⁸ these conflicting reports can be explained by variations in dantrolene sodium and caffeine concentrations and also by variation in the sensitivities of different muscle preparations to caffeine. If caffeine and dantrolene sodium are antagonistic at the same subcellular site(s), one would expect the caffeine-induced contracture response in MHS muscle to be at least attenuated by dantrolene sodium. Dantrolene sodium, in this study, did not prevent caffeine-induced contractures in MHS muscle fibers. This apparent paradox may be explained by the low concentration of dantro-

lene sodium used in this study, by the extreme sensitivity of MHS muscle to caffeine, and by the potentiation by halothane of caffeine-induced contractures.¹²

Since dantrolene sodium, at the concentration used in this study, conferred significant protection against and reversed halothane-induced contractures, and since dantrolene sodium produces muscle relaxation by inhibiting calcium release from the SR, it is possible that dantrolene sodium may block the effect of halothane on SR and/or mitochondrial membranes. Dantrolene sodium apparently ensures normal intracellular calcium concentration in MHS muscle in the presence of halothane.

Dantrolene sodium, unlike other clinically used muscle relaxants, appears to be effective in prevention and treatment of *in vitro* and *in vivo* halothane-induced malignant hyperthermia.

Succinylcholine appears to act synergistically with halothane in triggering the malignant hyperthermia syndrome in swine,¹⁴ while in man, succinylcholine may initiate the onset of malignant hyperthermia in the absence of halothane.² Succinylcholine also produces marked contractures in isolated human MHS

muscle.² The role of the nondepolarizing relaxants in malignant hyperthermia is less obvious but certainly, no absolute protection against malignant hyperthermia is associated with their use, and in fact several case reports have implicated these agents as possible triggering agents.²

The evidence from this study suggests that dantrolene sodium may be effective in a prophylactic and therapeutic role in MHS patients. Its preoperative use in patients known to be at risk for malignant hyperthermia may provide a safeguard during anesthesia of these patients.

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Obstetric Anesthesia

TRICHLOROETHYLENE FOR CESAREAN SECTION The authors report a comparison of 0.1 per cent trichloroethylene-N₂O-O₂ with 0.1 per cent methoxyflurane-N₂O-O₂ for cesarean section in 405 patients. The incidences of awareness and unpleasant dreams were 6.3 per cent with trichloroethylene and 3.5 per cent with methoxyflurane. There was no difference in the incidences of nausea, vomiting, headache, and status of the newborn infant. Of considerable importance was the unexpected finding that the clinical and biochemical condition of the infant did not correlate with the

time between induction of anesthesia and the initial incision into the myometrium. However, there was a direct and significant correlation between the infant's condition and the interval from incision of the uterus to completion of delivery. The authors conclude that "the critical period is that during which the uterus is being manipulated when, presumably, foeto-placental vascular dynamics are subjected to considerable interference." (*Crawford JS, Davies P: Return to Trichloroethylene for Obstetric Anaesthesia. Br J Anaesth* 47: 482-490, 1975.

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