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Porcine Malignant Hyperthermia:

Effects of Temperature and Extracellular Calcium Concentration on Halothane-induced Contracture of Susceptible Skeletal Muscle

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Skeletal muscle from malignant hyperthermic (MH) pigs incubated at 37 C in 2.3 mM calcium–Krebs-Ringer solution contracts spontaneously when exposed to halothane. In contrast, halothane did not induce contracture in MH muscle incubated in 2.3 mM calcium–Krebs-Ringer solution at 25 C or in calcium-free Krebs–Ringer's solution at 37 C. Halothane did not induce contracture in normal control muscle in 2.3 mM Krebs-Ringer solution at 25 or 37 C. In the presence of halothane, addition of caffeine produced greater contracture in MH muscle than in normal controls. Halothane–caffeine-induced contractures of MH and control muscles at 25 and 37 C were similar. Elucidation that under certain experimental conditions halothane induces contracture in MH muscle, but not in normal muscle 1) may aid in development of a diagnostic test; 2) establishes further evidence for skeletal muscle as the target tissue for anesthetic-induced MH; 3) suggests that halothane may affect systems that regulate sarcoplasmic calcium concentration below contracture threshold in MH muscle. (Key words: Hyperthermia, malignant; Anesthetics, volatile, halothane; Ions, calcium; Muscle, skeletal, malignant hyperthermia.)

ANESTHETIC-INDUCED malignant hyperthermia (MH) is often associated with abnormalities in skeletal musculature of susceptible man and pigs.[1–4] Much of the data on MH have indicated that genetically predisposed skeletal muscle is the target tissue for anesthetic agents that trigger MH. Skeletal muscle rigidity occurs in 70 per cent of MH patients[1] and occurred in 98 per cent of 91 related MH Poland China pigs studied in our laboratory (Nelson TE unpublished data).

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Abnormal responses of skeletal muscle *in vitro* have demonstrated direct effects of halothane on muscle from MH patients and pigs.[3–7] Muscles from MH pigs have lower adenosinetriphosphate (ATP) levels and produce more lactate when treated with halothane.[3,4] Skeletal muscle of MH patients is hypersensitive to caffeine-induced contracture *in vitro* and to halothane potentiation of this contracture.[5,7]

The findings of Ellis *et al.*[6] and Moulds and Denborough[7] that halothane induces spontaneous contracture in muscle from MH patients differ from those of Kalow *et al.*,[5] in which halothane alone did not induce contracture. Although halothane did not induce contracture of MH muscle in Kalow's subjects, it did inhibit calcium uptake by sarcoplasmic reticulum isolated from muscle of the patients who had rigidity with MH.[5]

Since the ability of halothane to induce contracture in MH-susceptible muscle is important from a diagnostic and etiologic viewpoint, we sought to determine whether halothane induces contracture *in vitro* in muscle from MH pigs. One difference we observed among the experimental procedures of investigators who did[6,7] and did not[5] obtain halothane-induced contractures in MH muscle lay in the temperatures used in the incubation solution. Kalow *et al.*[5] did not obtain halothane-induced contractures working at 25 C, whereas Ellis *et al.*[6] and Moulds and Denborough[7] obtained spontaneous contracture when MH muscle was exposed to halothane at 37 C. A loss in regulation of intracellular calcium concentration of MH muscle has been suggested as an etiologic factor for MH.[5,7] For this reason, it was considered important to determine whether varying extracellular calcium could affect the abnormal contracture response of MH muscle. The purpose of this study was to com-

pare the effects of temperature and extracellular calcium concentration on halothane and halothane–caffeine-induced contractures in muscle of MH pigs.

Methods

The purebred MH Poland China pigs used in this study are part of a colony of MH-susceptible pigs bred and maintained at this institution.[8] Three weeks prior to *in-vitro* testing, each pig was determined to be MH susceptible by the development of skeletal muscle rigidity and/or hyperthermia subsequent to administration of halothane or halothane plus succinylcholine. Purebred Hampshire pigs that did not develop MH when challenged with concurrent halothane and succinylcholine were used to provide a source of control muscle. Anesthesia was induced in each pig with thiopental, and, following intubation, the pig was challenged with 2 per cent halothane in oxygen. Pigs MH 19-3 and MH 18-6 developed typical fulminating MH with skeletal muscle rigidity after 15 and 40 minutes, respectively, of 2 per cent halothane anesthesia. Pig 19-3 recovered from MH when halothane was discontinued and respirations were assisted. Skeletal muscle rigidity persisted in Pig 18-6 for 35 minutes after halothane was discontinued. At this time, procaine, 20 mg/kg, was administered intravenously, and there was immediate reversal of the skeletal muscle rigidity. Since Pig MH 17-1 had no symptoms of MH after 2 per cent halothane for 1 hour; 2 per cent halothane was continued and succinylcholine (7 mg/kg), was administered intravenously, with consequent mild, transient fasciculations. After administration of succinylcholine and maintenance of Pig MH 17-1 on 2 per cent halothane, a slow increase in temperature of 0.7 C occurred in the ensuing 1.5 hours, after which rigidity was observed; halothane was discontinued and the pig had an uneventful recovery.

For the study of contracture *in vitro*, bundles of muscle fiber were dissected from the gracilis muscles of each pig after it was anesthetized with thiopental and the trachea intubated and while respirations were assisted with 100 per cent O_2. Thiopental can be used for induction and maintenance of anesthesia for pigs,[9] and was the sole anesthetic agent used for muscle biopsy in this study. Each bundle of fibers was tied at resting length to applicator sticks, cut at each end, and placed immediately in Krebs-Ringer (KRB) solution at either 25 or 37 C. Muscle fibers to be tested in calcium-free KRB solution were placed in calcium-free KRB solution at 37 C. The bicarbonate-buffered (*p*H 7.4) Krebs-Ringer solution contained (in millimoles/liter): NaCl 118; $NaHCO_3$ 24.6; KCl 4.6; $CaCl_2$ 2.31; KH_2PO_4 0.77; $MgSO_4$ 0.77. A 25-mM caffeine solution was prepared in Krebs-Ringer solution and at the designated time a 5-ml amount was added to 40 ml of reaction solution to obtain a final concentration of 2.8 mM. All solutions were bubbled continuously with carbogen (a mixture of 95 per cent O_2 and 5 per cent CO_2). Halothane, when required, was introduced by flowing 2 l/min carbogen through a Fluomatic vaporizer. The bundles of muscle fibers averaged 4 cm long, 2 mm wide, 1 mm thick, and 130 mg in weight. Each bundle was subsequently removed from the applicator stick and one end attached to a hollow glass suspending rod, which also served as a means of bubbling carbogen into the solution. The suspension rod with one end of the muscle attached was placed in a 50-ml syringe barrel containing 40 ml KRB solution and secured by a clamp. The free end of the muscle was attached by silk thread to a microscale accessory attached to a Statham model UC4 force–displacement transducer for measuring isometric tension. The transducer signal was amplified and recorded by a Beckman Dynograph. An initial tension of 1 g was placed on each muscle and carbogen was bubbled through the solution for 5 minutes. This was followed by a series of treatments lasting 5 minutes each, in the following sequence: a) 1 per cent halothane; b) 2 per cent halothane; c) 3 per cent halothane; d) 4 per cent halothane; e) 5 per cent halothane; f) 5 per cent halothane and 2.8 mM caffeine. Changes in muscle tension during each 5-minute period reached steady-state levels, and tension was measured either as the change from one steady-state level to the next or as cumulative

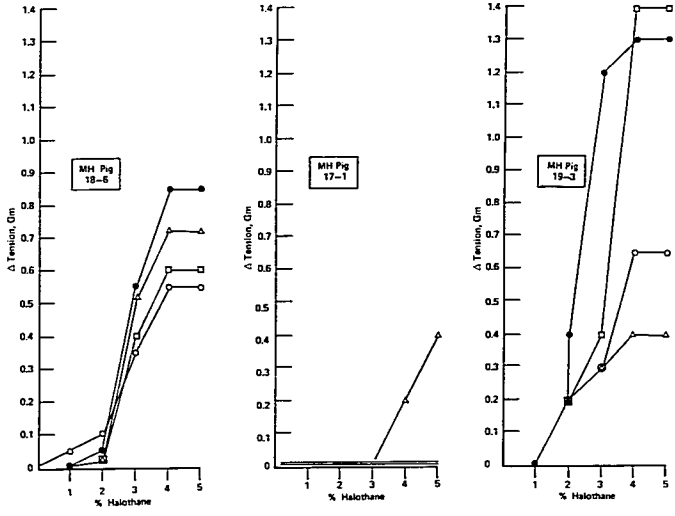

FIG. 1. Halothane-induced contractures in MH muscle fibers. Each symbol represents one of four muscle fibers tested for each pig. Only one of four muscle fibers from Pig MH 17-1 responded to halothane with contracture.

tension. Each pig was biopsied twice, initially for the 37-C study and a second time for the 25-C study. Four fibers were prepared from each of three MH Poland China and three control Hampshire pigs for each of the two temperature studies. The addition of 2.8 mM caffeine at the end of halothane testing was instituted as a test for the ability of the muscle fiber preparation to respond with contracture. Student's t test was utilized for statistical evaluation of the data.

Results

Halothane in concentrations of 1 to 5 per cent in carbogen did not induce contracture in any of the normal control muscle fibers tested at 25 and 37 C or in any of the MH muscle fibers tested at 25 C. Contracture responses of MH muscle fibers to halothane occurred when the incubation temperature was 37 C. Halothane induced contractures at 37 C in all muscle fibers from two of the three MH pigs studied (MH 18-6 and MH 19-3), while only one of four fibers from MH Pig 17-1 contracted in response to halothane treatment (fig. 1). In most MH fibers (6 of 9) tension was induced at 2 per cent halothane concentration and tension increased linearly with halothane concentrations of 2, 3, and 4 per cent. The effect of calcium-free KRB solution at 37 C on the halothane-induced contracture of MH muscle is illustrated by the data of figure 2. Halothane in concentrations of 1 to 5 per cent in carbogen did not induce contracture in any of 12 MH fibers or 12 normal fibers tested in calcium-free KRB solution at 37 C. Calcium-free KRB solution also reduced contracture with 5 per cent halothane plus 2.8 mM caffeine; contracture was restored by calcium repletion (fig. 2).

MH fibers had greater cumulative contracture responses to 5 per cent halothane plus 2.8 mM caffeine than did the control fibers

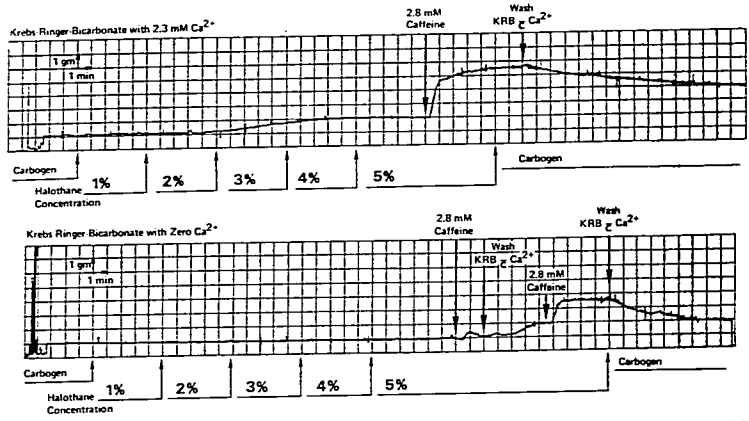

FIG. 2. The top trace shows halothane-induced contracture of MH muscle in KRB containing 2.3 mM Ca^{++}. After 5 per cent halothane, addition of 2.8 mM caffeine caused additional contracture, which was partially reversed by KRB wash. The bottom trace shows the loss of halothane–caffeine-induced contracture when MH muscle was incubated in KRB without Ca^{++}. After 5 per cent halothane + 2.8 mM caffeine, a wash with KRB containing 2.3 mM Ca^{++} restores the contracture response that was reversed by the KRB wash.

(table 1). Temperature had no effect on the contracture responses to 2.8 mM caffeine plus 5 per cent halothane in either MH or control muscle fibers (table 1). Compared with halothane–caffeine-induced contractures at 37 C in 2.3 mM calcium–KRB solution, contractures in calcium-free KRB solution were reduced 91 per cent in MH muscle and 78 per cent in normal muscle (table 2). Muscle fibers of MH Pig 17-1, which appeared less sensitive to halothane-induced contracture, had lower caffeine–halothane-induced contractures compared with fibers from Pigs MH 18-6 and MH 19-3 (table 1).

In contrast to halothane–caffeine-induced contracture of MH pig muscle, halothane-induced contracture is of lower magnitude, is dependent on temperature and extracellular calcium concentration, and has a maximum concentration effect below maximal contracture response.

Discussion

The results of this investigation show the temperature dependence of halothane-induced contractures in MH muscle. This finding offers an explanation for the apparently conflicting results obtained by Kalow *et al.*,[5] which indicated that halothane does not induce contracture of MH muscle exposed at 25 C, and Ellis *et al.*[6] and Moulds and Denborough,[7] in which halothane induced contracture in muscle of MH patients when treated at 37 C. It appears that under the appropriate experimental conditions halothane does cause contracture responses in MH but not in normal skeletal muscle.

The effects of lowering temperature and extracellular calcium concentration on the abnormal contracture response of MH muscle to halothane and halothane plus caffeine provide additional information regarding possible sites of action for halothane. In the resting state of muscle, it estimated that the sarcoplasmic free calcium concentration is less than 10^{-7} M, and when free calcium concentration increases to above this level, muscle contraction is initiated.[9] The source of calcium for contraction response to membrane depolarization or for contracture in response to caffeine treatment is generally

TABLE 1. Effects of Temperature on Contractures Induced by 2.8 mM Caffeine and 5 Per Cent Halothane Treatment of MH and Control Muscle Fibers

Source of Muscle	Cumulative Tension (g)*		t Test 37 vs. 25 C
	At 37 C	At 25 C	
MH pig 17-1	7.5 ± 1.2† n = 4	6.2 ± 0.38 n = 4	N.S.
MH pig 18-6	10.8 ± 2.1 n = 4	11.3 ± 1.1 n = 4	N.S.
MH pig 13-3	11.3 ± 0.5 n = 4	12.0 ± 0.87 n = 4	N.S.
Average, MH pigs	9.9 ± 0.91 n = 12	9.8 ± 0.90 n = 12	N.S.
Control pig 4-3	2.8 ± 0.34 n = 4	2.7 ± 0.36 n = 4	N.S.
Control pig 22-10	1.9 ± 0.19 n = 3	1.8 ± 0.29 n = 4	N.S.
Control pig 27-7	3.1 ± 0.52 n = 4	4.0 ± 0.69 n = 4	N.S.
Average, control pigs	2.7 ± 0.25 n = 11	2.8 ± 0.36 n = 12	N.S.
t Test, MH *vs.* control	$P < 0.01$	$P < 0.01$	

* Tension shown is total change in tension after treatment sequence of 1, 2, 3, 4, and 5 per cent halothane, and 2.8 mM caffeine plus 5 per cent halothane (see text for details).
† Values are means and standard errors; n is the number of fibers tested.

TABLE 2. The Effect of Calcium Concentration on Contractures Induced by 2.8 mM Caffeine and 5 Per Cent Halothane Treatment of MH and Control Muscle Fibers at 37 C

	Cumulative Tension (g)*		t Test 2.3 mM Ca^{++} *vs.* Ca^{++} Free KRB
	2.3 mM Ca^{++} KRB	Ca^{++} Free KRB	
MH muscle	9.9 ± 0.91† n = 12	0.88 ± 0.70 n = 10	$P < 0.001$
Control muscle	2.7 ± 0.25 n = 11	0.60 ± 0.38 n = 7	$P < 0.001$
t Test, MH *vs.* control	$P < 0.001$	$P < 0.001$	

* Tension shown is total change in tension after treatment sequence of 1, 2, 3, 4, and 5 per cent halothane, and 2.8 mM caffeine plus 5 per cent halothane (see text for details).
† Values are means and standard errors; n is the number of fibers tested.

accepted to be that calcium stored in the terminal sacs of the sarcoplasmic reticulum.[10] The loss of halothane-induced contracture of MH muscle at 25 C may be related to a temperature-dependent calcium flux from either an intracellular or an extracellular source. Since the halothane–caffeine-induced contracture is not affected by lowering the temperature from 37 to 25 C, it appears that the flux of calcium from the intracellular sarcoplasmic reticulum source is not affected by this temperature difference. The loss of halothane contractures when MH muscle is incubated in calcium-free KRB solution at 37 C may relate to a loss of stored calcium from the muscle, or loss of a halothane-sensitive mechanism that is coupled to calcium release. The marked

decrease in halothane–caffeine-induced contractures when muscle is incubated in calcium-free KRB is compatible with a loss of calcium from storage sites, since caffeine-induced contracture does not require a coupled system.[11] A rapid exchange of calcium between extracellular fluid and intracellular halothane–caffeine-sensitive pools is apparent from the contracture response obtained when the calcium-free KRB is replaced with 2.3 mM calcium–KRB solution. The temperature dependence of halothane-induced contracture of muscle and the extracellular-calcium-concentration dependence of halothane- and halothane–caffeine-induced contractures of MH muscle may be associated with an effect of halothane on the influx of calcium from the extracellular fluid.

The fact that halothane induces contracture in MH muscle, but not in normal muscle, may be useful in identifying susceptibility to malignant hyperthermia.

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Erratum

Two errors appeared in the article by Trichet *et al.*, "The Effect of Pre-existing Pulmonary Vascular Disease on the Response to Mechanical Ventilation with PEEP Following Open-heart Surgery" (ANESTHESIOLOGY 42:56–67, 1975). On page 56, the sentence beginning in the sixth line of the second column should read: "Following AVR, $\dot{Q}_s/\dot{Q}_t$ changed in the same direction as cardiac index (CI) irrespective of ventilatory pattern: $\dot{Q}_s/\dot{Q}_t$ decreased as CI diminished and rose as CI increased." On page 57, the sentence beginning in the eighth line below Table 1 should read: "Cardiac output was determined by the dye-dilution technique using 5 mg indocyanine green dye with injection into the pulmonary artery and withdrawal from the radial artery into the densitometer (Beckman Cardiodensitometer, Model SP284 and SP305)."